IOWA STATE COLLEGE

JOURNAL OF SCIENCE

Published on the first day of October, January, April and July.

All manuscripts submitted for publication should be addressed to R. E. Buchanan, Room 101 Science Building, Iowa State College, Ames, Iowa.

All remittances should be addressed to P. E. Brown, Bus. Mgr., Room 25 Hall of Agriculture, Iowa State College, Ames, Iowa.

Single Copies: One Dollar; Annual Subscription Three Dollars; In Canada, Three Dollars and a Quarter; Foreign, Three Dollars and a Half.

Entered as second class matter at the Post Office, Ames, Iowa.

ALKYL-ARYL LEAD COMPOUNDS. ANTI-KNOCK STUDIES

By HENRY GILMAN, O. R. SWEENEY AND J. E. KIRBY

From the Departments of Chemistry and of Chemical Engineering at Iowa State College.

Accepted for publication July 17, 1928.

INTRODUCTION

Tetra-ethyl lead is at present the most widely used anti-knock reagent. However, unsaturated and aryl groups apparently increase the anti-knock effectiveness of various gasolines. It is for this reason, in part, that an added emphasis is being placed by some on the superior qualities of gasoline obtained by cracking processes[1]. If this idea is sound, then it is reasonable to expect that organolead compounds formed by replacing some or all of the saturated ethyl goups in tetra-ethyl lead by unsaturated or aryl groups should be more effective anti-knock agents than tetra-ethyl lead. Accordingly, a study is being made of such compounds[2].

The compounds reported here are the *n*-butyl, *iso*-butyl, *sec.*-butyl and *tert.*-butyl, triphenyl leads. The preparation of *tert.*-butyl triphenyl lead is unusual. Hitherto, "no lead compound containing a tertiary hydrocarbon group has been prepared. All the attempts have resulted only in a reduction with deposition of metallic lead"[3]. Related reduction reactions were observed in the first attempts to prepare *tert.*-butyl triphenyl lead. For example, when triphenyl-lead bromide was added to *tert.*-butyl-magnesium chloride, a mixture resulted from which only the highly interesting triphenyl-lead (or hexa-phenyl di-lead) could be isolated. However, by reversing the order of addition by adding the Grignard[4] reagent to triphenyl-lead bromide, the desired compound was obtained. With *tert.*-butyl-magnesium chloride in excess, the Grignard reagent may act as a reducing agent according to the following reaction:

$$tert.\text{-}C_4H_9MgCl + (C_6H_5)_3PbBr \rightarrow C_4H_8 + C_4H_{10} + (C_6H_5)_3Pb + MgClBr$$

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[1] One of the most recent and leading accounts of petroleum and its products is by Burrell, *Ind. Eng. Chem.*, 20, 602 (1928).

[2] This is part of a more general study of organolead compounds, particularly with a view to their application in the treatment of cancer and some related plant diseases. See, Gilman and Robinson, *J. Am. Chem. Soc.*, 49, 2315 (1927) and 50, 1714 (1928).

[3] Calingaert, "Organic Compounds of Lead," *Chemical Reviews*, 2, 43 (1926). This is the most recent and authoritative treatise on organolead compounds. Prior to the preparation of *tert.*-butyl triphenyl lead, Dr. Balassa prepared di-*tert.*-butyl-diphenyl lead in this Laboratory.

[4] One of the general methods for the large-scale production of tetra-ethyl lead involves the use of the Grignard reagent.

Gilman, Sweeney and Beaber[5] prepared tetra-phenyl lead in large quantities by means of the Grignard reaction and tested its anti-knock properties and its solubility in nitrobenzene, inasmuch as such nitro compounds have distinct anti-knock properties and were for a time sold as anti-knock agents. Their comparative tests indicated that tetra-phenyl lead had distinct and promisingly superior qualities. The compounds described in the present report are, however, being tested with a series of related compounds by another method in a comprehensive study concerned with the correlation of chemical constitution and anti-knock effectiveness. By this method, organolead compounds that are solid at room temperatures and sparingly soluble in gasoline cannot be tested with any great reliability. However, related organolead compounds now being tested and having a lesser number of aryl groups indicate that the anti-knock effectiveness increases somewhat with the branching of radicals.

EXPERIMENTAL

The tetra-phenyl lead used in these studied was prepared according to the method of Pfeiffer and Truskier[6]. Subsequently, Gilman and Robinson[7] devised improved directions for its preparation from the Grignard reagent and lead chloride.

Triphenyl lead bromide was prepared according to the method of Grüttner[8]. It is essential that pure pyridine be used in this low temperature (—50°) bromination. With the use of pure pyridine and in runs one-half the size of that described by Grüttner, the yields were 80% or better.

n-Butyl-Triphenyl Lead. $C_4H_9Pb(C_6H_5)_3$.—A solution of 0.08 mole of *n*-butylmagnesium bromide was prepared from 11 g. of *n*-butyl bromide and 1.95 g. of magnesium. This solution was diluted to about 250 cc. with dry ether and then 20 g. (0.04 mole) of triphenyl lead bromide was added in small quantities. A slight heat of reaction was noticed. The reaction mixture was stirred and gently refluxed about one and one-half hours and then hydrolized by pouring on iced ammonium chloride solution. A little ammonium hydroxide was added and the entire mixture was filtered with suction to remove a small quantity of dark insoluble material. The ether layer was removed, dried over sodium sulfate, and then concentrated. The yellowish paste that resulted became solid when rubbed with alcohol. It was washed twice with 95% alcohol and air-dried to constant weight. The yield was 12.7 g. or 66.5% of the theoretical amount. When once recrystallized from 95% alcohol the white crystals melted sharply at 47°.

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[5]Doctorate Thesis of N. J. Beaber, Iowa State College, 1925. In the article by T. A. Boyd, "Quantitative Effects of Some Compounds in Detonation in Internal Combustion Engines" (*International Critical Tables*, volume 2, pages 162-163), there is no mention made of nitro compounds in his list of some anti-knock compounds. Also, the tetra-phenyl is rated there as about 50% per mole, as effective as tetra-ethyl lead.

[6]Pfeiffer and Truskier, *Ber.*, 37, 1123 (1904).

[7]Gilman and Robinson, *J. Am. Chem. Soc.*, 49, 2315 (1927).

[8]Grüttner, *Ber.*, 51, 1298 (1918).

Analysis.—All of the organolead compounds reported here were analyzed for lead by the method described recently by Gilman and Robinson[9]. *Calc.* for $C_{22}H_{24}Pb$: Pb, 41.82%. *Found*: Pb. 41.46 and 41.52%.

Iso-Butyl Triphenyl Lead.—The procedure was the same as the one just described for the preparation of *n*-butyl triphenyl lead. The yield was 15.4 g. or 80.6% of the theoretical amount. When twice recrystallized from alcohol the compound is obtained as fine needles melting sharply at 68-68.5°.

Analysis.—*Calc.* for $C_{22}H_{24}Pb$: Pb, 41.82%. *Found*: Pb, 41.38 and 41.21%.

Sec.-Butyl Triphenyl Lead.—This compound was prepared according to the directions for the preparation of the two preceding compounds. The yield was 12.3 g. or 64.4% of the theoretical amount. After one recrystallization from 95% alcohol the compound melted sharply at 84°.

Analysis.—*Calc.* for $C_{22}H_{24}$ Pb: Pb, 41.82%. *Found*: Pb, 41.59 and 41.43%.

Tert.-Butyl Triphenyl Lead.—A solution of 0.2 mole of *tert.*-butylmagnesium chloride was prepared according to the improved directions described recently by Gilman and Zoellner[10] from 18.4 g. of *tert.*-butyl chloride and 5 g. of magnesium turnings. To this solution was added 19.5 g. of triphenyl lead bromide. The heat of reaction was very slight and the solution had a yellowish color. The insoluble material obtained subsequent to hydrolysis with iced ammonium chloride and ammonium hydroxide was filtered by suction and washed with ether. The yellow colored ether layer and washings were dried over sodium sulfate and then concentrated to give a yellowish solid that weighed 8.6 g. and melted unsharply at 95°. Recrystallization from alcohol and then from benzene gave yellow crystals that melted unsharply and with decomposition at 150°.

The mother liquor and washings were diluted with alcohol and on standing two distinct types of crystals separated: flat, white plates and star-like clusters of yellow needles. These crystals were filtered, dried and then carefully separated by hand. The white crystals correspond closely with triphenyl lead obtained by Krause and Reiszaus[11] who have described triphenyl lead as crystallizing from benzene in light yellow crystals which begin to melt at 155° and are completely melted at 225°, the melt being colored black with free metallic lead. These crystals contain 1.5 molecules of benzene. When the benzene is replaced by alcohol a compound is obtained which is nearly white and contains no solvent of crystallization. Their compound gave a green coloration when dissolved in benzene and treated with alcoholic silver nitrate. The white compound obtained in our experiment begins to decompose at 148° and at 220-222° breaks down to a gray mass. It also gives the green color-test with alcoholic silver nitrate.

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[9]Gilman and Robinson, *J. Am. Chem. Soc.*, **50**, 1714 (1928).

[10]Gilman and Zoellner, *ibid.*, **50**, 425 (1928).

[11]Krause and Reiszaus, *Ber.* **55**, 888 (1922).

This color test was found to be negative with *n*-butyl triphenyl lead and with triphenyl lead bromide.

Analysis.—Calc. for $C_{18}H_{15}Pb$: Pb, 47.26%. *Found*: Pb, 47.33%. The yellow crystals were not identified. They melt at 95-97° to an opaque yellow mass, with a red color which appears at 110°, turning to black at 112°. These crystals also give the silver nitrate color test.

In a second experiment the order of addition was reversed, the *tert.*-butylmagnesium chloride being added slowly to 10.4 g. or 0.02 mole of triphenyl lead bromide suspended in ether. The Grignard solution was prepared from 4.6 g. or 0.05 mole of *tert.*-butyl chloride, 1.22 g. of magnesium turnings and 70 cc. of ether. In order to minimize the supposed reducing reaction of the Grignard reagent, only one-half of this RMgX solution was added; and, inasmuch as the yield of *tert.*-butylmagnesium chloride was only about 65%[10] the effective quantity of Grignard reagent added was less than the theoretical amount necessary for a complete reaction. This undoubtedly accounts in part for the low yield and for the difficulty encountered in the separation of the reaction product from unaltered triphenyl lead bromide.

During the addition of the Grignard solution a yellow color appeared. The mixture was hydrolyzed with iced ammonium chloride and filtered. On concentrating the yellow ether layer by evaporation a yellow solid resulted, and this underwent decomposition on heating with alcohol. This material was not further investigated. The residue from filtration was extracted with several small portions of hot alcohol, and on cooling white needles separated. Repeated crystallization from alcohol gave glistening needles that melted sharply at 150-150.5°. When mixed with a sample of triphenyl lead bromide (melting at 157°) the melting point was depressed to 130°.

Analysis.—Calc. for $C_{22}H_{24}Pb$: Pb, 41.82%. *Found*: Pb, 41.60 and 41.69%.

SUMMARY

The four butyl triphenyl leads have been prepared from the appropriate Grignard reagent with triphenyl lead bromide. Some triphenyl lead or hexaphenyl di-lead was obtained in connection with the preparation of *tert.*-butyl triphenyl lead. This *tert.*-butyl derivative, apart from its value in anti-knock studies, is the first reported organolead compound having a tertiary radical attached directly to lead.

THERMAL CONDUCTIVITIES OF GLASSES TRANSMITTING ULTRA-VIOLET LIGHT

WM. KUNERTH AND WM. E. BERKEY

From the Department of Physics, Iowa State College

Accepted for publication September 15, 1928

Increased knowledge of the beneficial therapeutic effects of ultra-violet light upon living organisms has in recent years led to the development of a number of glasses which transmit ultra-violet light more or less completely. Among them may be mentioned Quartz-lite, Corex, Vita glass and Helio glass. It is evident that if any of these are to replace the window glass now being used, they must be very poor conductors of heat, for otherwise we might pay dearly for the benefits secured by the use of these glasses because of the greater amount of heat they would allow to escape from a room by conduction. It therefore becomes important to determine the thermal conductivity of the glasses under consideration.

Since the thermal conductivity of glass is very low, it is impossible to get reliable results by the use of the ordinary laboratory method of determining conductivity. The method here resorted to was originally due to Christiansen. If a slab of a given material of thickness d, with parallel faces each of area A, has its opposite faces kept at temperatures t_1 and t_2, respectively, the quantity of heat, Q, which will pass by conduction through the slab from one face to the other in a time T is directly proportional to the area of the face, the difference in temperature, and the time, and inversely proportional to the thickness.

$$\text{Then } Q = \frac{k\ A(t_2 - t_1)\ T}{d}$$

In this expression k is a constant known as the thermal conductivity of the substance.

If two flat plates are placed in contact, face to face, and a steady flow of heat is established between them, perpendicular to the broad face, the heat Q which passes in time T is

$$Q = \frac{k_1\ A\ (t_2 - t_1)\ T}{d_1} = \frac{k_2\ A\ (t_3 - t_2)\ T}{d_2}$$

$$\text{or } \frac{k_1}{k_2} = \frac{d_1}{d_2} \cdot \frac{(t_3 - t_2)}{(t_2 - t_1)}$$

This means that the ratio of the conductivities is given by the ratio of temperature differences and thicknesses. The actual quantity of heat need not be measured. If k_2 is the conductivity of the standard of com-

parison, k_1 may be determined if we have the thicknesses and temperatures given.

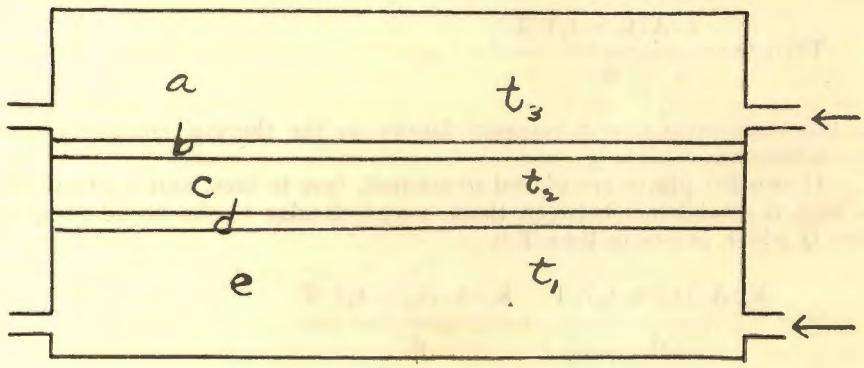
In the figure a is a steam chest through which live steam is passed, b is a layer of a substance whose thermal conductivity is known, c is a copper plate into which a thermometer is inserted to get the temperature t_2, t_3 is the temperature of the steam, t_1 that of the water, d is the test material, and e is a water chest through which cold water is passed.

The conductivity of bakelite which was used for layer b may be taken as 0.00037 c.g.s. units. Glycerine was placed between any two consecutive layers to insure good thermal contact. Steam and water must be passed continuously for two hours. The copper plate, c, has a very high conductivity and the temperature gradient through it may be neglected.

The results obtained for the thermal conductivities were as follows:

Kind of Glass	Thermal Conductivity
Quartz-lite	0.000481
Corex	0.000512
Helio glass	0.000565
Vita glass	0.000602
Plain window glass	0.000716

Ordinary window glass does not transmit ultra-violet light. Experiment shows that it is a better conductor of heat than the other glasses tested. There are therefore at least two reasons why the window glass now being used should be replaced by other glasses; first, because the glass substitutes transmit some ultra-violet light; and second, because they allow less heat energy to escape by conduction.



STUDIES IN HOME CANNING*

III. *Heat Penetration in Meats and Vegetables Processed in Glass Containers***

GAIL M. REDFIELD, P. MABEL NELSON AND GERTRUDE SUNDERLIN

From the Department of Foods and Nutrition, Iowa State College

Accepted for publication Sept. 15, 1928.

This work was undertaken as part of a study (26) (27) to formulate more accurate time tables than those available at present for the canning of the non-acid vegetables and meats in the water bath and pressure cooker under home conditions.

An enormous literature relating to the canning of food products, stimulated by the need for improved technique, has been developed in the last few years. Most of the experimental work has dealt with phases other than the rate of penetration of heat into jars, probably because of the difficulty of securing an exact record of the temperature of the interior of the jar and of the retort during processing.

Earlier experiments as those of Belser (5) showed, by the use of maximum thermometers in the jars, the maximum temperature attained in a given time, in some of the common fruits and vegetables. Beveridge and Fawcus (6) used thermometers for a study of the heat penetration in meat canned in tin.

The work of Kochs and Weinhausen (20) in Germany was similar to that of Belser. As the original of their work was not available, only the comment of Bigelow, et al (9), on this work can be given. It is as follows:

"In 1906 Kochs and Weinhausen gave the results obtained in a study of heat penetration in tin cans and glass and earthenware jars. Maximum thermometers of special construction were used and were held in position by being fastened to the side of the container. The results, therefore, gave only the maximum temperature attained at the center of the can and gave no information regarding the temperature at various intervals. The results are of little value and the work was quite crude, but it is of interest as pointing to a recognition of the necessary of information on the subject."

Later thermocouples were used for the study of heat penetration by Bovie and Bronfenbrenner (11). They undertook the problem of determining the rate of heat transfer from the outside toward the center of cans of food, during the process of cooking and sterilization, as affected by the variations of the autoclave, temperature, size of can and viscosity of the food.

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*The data in this paper are taken in part from a thesis submitted by Gail M. Redfield in partial fulfillment for the degree of Master of Science, Iowa State College.

**Funds furnished through Ball Brothers Company, Muncie, Indiana.

Thompson (28) determined, by means of thermocouples, the temperature-time curves when cans are subjected to various temperatures in hot water, steam, cool air and cool water. He used fruits and vegetables in order to get as great a variety of conditions as practicable. He found that those products requiring a large amount of free liquid to fill the cans permit considerable convection currents and those requiring no liquid permit practically none. Ball (2) (3) made extensive studies of the mathematical relationships involved in heat penetration and sterilization of canned foods.

A summary of the findings of Bigelow and his co-workers (9) and of Magoon and Culpepper (21) (22) will be given, as the result of their work has the most direct bearing on this problem.

Bigelow, et al, found:

1. That heat penetration is most rapid in products that consist of or are surrounded by water, or a thin syrup or brine.
2. That products as peas, which consist of small round particles that are not cooked to pieces, permit the movement of heat by means of convection currents almost as quickly as water.
3. That products which soften when heated and packed together, and products which are cooked to pieces during the process, make the solution somewhat viscous and retard heat penetration. If the pieces of insoluble material are somewhat larger like beets and large plums they delay the heating of the liquor, which does not reach retort temperature until the pieces are heated to the center.
4. That there appears to be no appreciable difference in the heat penetration of cans processed under water and those processed in dry steam if the conditions are otherwise the same.
5. That the temperature is found to be the same in all parts of the retort.
6. That the heat penetration of canned foods is governed largely by the freedom with which convection currents are formed.
7. That the maximum heat penetration of canned food is that of water or perhaps slightly less. The minimum heat penetration is that of a body consisting largely of water, but in which the water is distributed in minute cells that entirely prevent convection.

Magoon and Culpepper (21) (22) showed:

1. That the factors affecting the rate of change of temperature at the center of the can are the diameter of the container, the conductivity, thickness and radiative power of the walls, the temperature conductivity, and mobility of its contents, and the temperature, conductivity and movement of the medium surrounding it.
2. That in a can packed with material having an interspace filled with a free liquid as in string beans, the rate of change of temperature at the center of the can is very rapid, and in materials of a heavy or pasty nature, as in sweet corn, the rate of heat penetration is very slow unless mechanical agitation is employed.
3. That the character of the pack and the composition of the material very largely determine the rate of change of temperature in the can.
4. That sodium chloride has very little direct effect upon the rate of change of temperature in the can. Dilute sugar solutions have only a small effect, concentrated solutions have a considerable effect in retarding

the rate of change. Materials of a viscous nature retard the rate of heat penetration.

5. That glass containers have a marked retarding effect upon the rate of rise in temperature in those materials in which there is a free liquid, as in string beans, but are of little importance in materials of a heavy consistency as sweet corn. Glass cools faster in air than tin, owing to its greater power of radiation.

6. That a proper temperature maintained for a length of time sufficient to prevent the subsequent development of the organisms causing the spoilage must be provided for first, but for the sake of the quality it should not be prolonged beyond the time essential to insure the keeping of the product and the safety of the food for human consumption.

7. That processing shall begin at the earliest possible moment after the preliminary treatment, otherwise time-temperature curves at the center of the can may actually fall during the first part of the processing period.

8. That with water, the rate of change of temperature at the center of the cans is very rapid when the external medium is water and very slow when it is air.

9. That in foods in which a free liquid fills the interspaces, the rate of change of temperature is very rapid, but while the maximum temperature is reached promptly, the maximum pressure is never reached during the ordinary processing periods, but continues to rise slowly as long as the high retort temperatures are maintained.

10. That in cans filled with material of a heavy consistency, the rate of change of temperature at the center of the can is very rapid at first and then becomes slower after the first few minutes. An equilibrium of pressure apparently is never reached, since in experiments where processing was continued for several hours, the pressure continued to rise as long as the retort temperature was maintained.

Other work of interest in this connection is that of Dugdale (14) (15), Bidault (7), Fellers (16), Fellers and Parks (17), Savage and Hunwicke (24) and Zavalla (29). Inasmuch as none of this work was as extensive as that previously mentioned, it will not be reviewed.

The above findings pertain to commercial processing of food products. The results obtained, however, have a direct bearing on home processing of foods. The studies of heat penetration most nearly comparable to home conditions are those of Castle (12), Bauer (4), and Denton (13).

Bitting (10) in a study of heat penetration in glass jars determined that heat penetration curves show a lag of 8° F. in a tin can and 20° in a glass jar below that of the surrounding bath. As a cover on the bath may make a difference of 30° much spoilage in home canning may be attributed to the use of an open bath.

Since this study was started Shank (25) and her associates have made an investigation of heat penetration in glass jars in oven canning and Gray (18) reported on heat transmission in glass packed products as compared to tin. Gray determined that when the ratio of the rate of heat through the container to the rate of heat through the product is greater than unity it does not matter whether glass or tin is used. When this ratio is less than unity the rate of heat penetration is somewhat faster in tin. When water was used the maximum temperature was reached 3 minutes

Water Bath Set-Up Showing Boiler, Jars with Thermo-Couples, Switch and Potentiometer in Position During Cooling.

FIGURE 1

sooner in tin than in glass, while with sweet corn no difference in time was noted.

In the present investigation the heat penetration in glass pint and quart jars containing various vegetables and meats packed under different home conditions and processed in the boiling water bath and the pressure cooker was studied.

MATERIAL AND METHODS

The equipment* consisted of a specially constructed ten point potentiometer with a switch and thermocouples which were devised for immersion in the water bath. The thermocouples were of copper and constantan wire soldered into Mason jar caps so that the point reached the center of the jar. Copper and constantan wires led from the caps to the potentiometer by means of which the temperature was read. In order to get the wires through the pressure cooker and still have the cooker steam tight, a stuffing box was put through the side of the cooker with metal and rubber discs, which, when forced together, made the connection steam tight. The cover was removed from the pressure cooker and the jars were left in the cooker during the cooling period. The wires used in the hot water bath and leading from the jars to the potentiometer were long enough to allow the jars to be set out of the bath for cooling without removing the caps.

The thermocouples were fastened to both pint and quart Mason jar caps. Glass pint and quart jars were used.

In both water bath and pressure cooker, the heating period was continued until the temperature at the center of the jar became constant, as evidenced by a cessation of rise of temperature for three consecutive readings at five minute intervals.

The readings of temperatures in the water bath were taken at five minute intervals and those in the pressure cooker at two and one-half minute intervals during the period in which the rate of rise of temperature was most rapid.

By the term processing temperature which is used in this paper is meant the temperature at the center of the jar which was considered as processing temperature when it approached within two degrees Fahrenheit of the temperature of the boiling water bath. It was considered that this two degree margin in temperature was within the limits of experimental error. It is understood that processing starts at lower temperatures, but for convenience of comparison the processing temperature was thus arbitrarily defined.

Records of rise in temperature are to be found in the thesis (23), which is on file in the library at Iowa State College.

RESULTS

BEEF AND PORK

There are many ideas prevalent at the present time regarding the technique of canning meat. Some people advocate putting a piece of bone into each jar, others claim that there is an advantage in adding fat with the meat, and still others claim that meat keeps better when it is precooked before being put into the jar.

*Designed by Leeds & Northrup, Philadelphia, Pa.

In order to determine the rate of heat penetration into meat, such variations in the pack as mentioned were used. Beef and pork were selected as examples of a lean and fat meat. It was thought that the results obtained with these meats would be applicable to the canning of other meats.

Beef round cut into convenient sized pieces was packed into the jar as cut; with water added, with fat added, with the addition of bone, and after precooking.

The initial temperature of the content of the jars varied with the differences in the preliminary treatment of the beef. The results obtained in the individual experiments with beef are depicted in Figure 2, which compares the rate of heat penetration in the different packs.

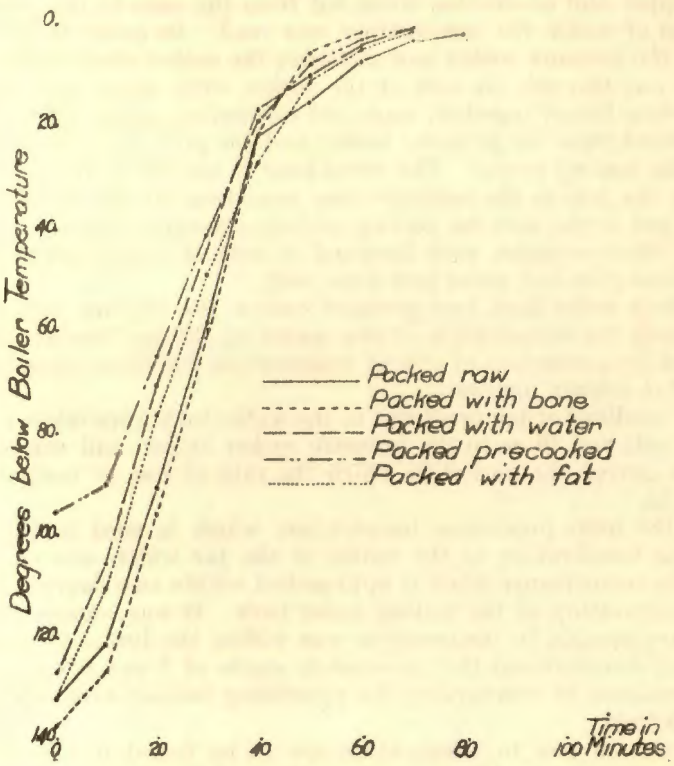

Figure 2. Heat penetration in beef packs in hot water bath.

From this record it will be seen that the beef with the bone heated most quickly to a constant temperature, due, probably, to the fact that the bone in the center of the jar allowed for greater convection currents. The precooked beef heated at a slower rate, due, probably, to the hardening of the fibers during precooking, which made the heat penetrate into the pieces of beef more slowly during the processing. Because of a higher initial temperature, the processing point was reached sooner with the precooked beef than with the beef packed raw. The beef with added fat heated a little

more slowly than the plain beef or than that with added water. The plain beef heated more slowly than the beef with added water, which corroborates the statement of Magoon and Culpepper (21) that in foods in which a free liquid fills the interspaces, the rate of change in temperature is more rapid due to the freedom with which convection currents are formed when it is heated.

Special pack beef round and beef suet—In order to test the heat conductivity of the fat of beef versus that of the lean of beef, two jars were packed solidly with beef suet, and two with a large piece of beef round in the center of the jar and smaller pieces packed around the sides of the jar to make the pack as solid as possible. They were then processed in the hot water bath.

The rate of heat penetration was more rapid into the jar of beef than into the jar of suet until the melting point of the suet was reached. After that point the temperature in the jar of suet rose almost as quickly as if

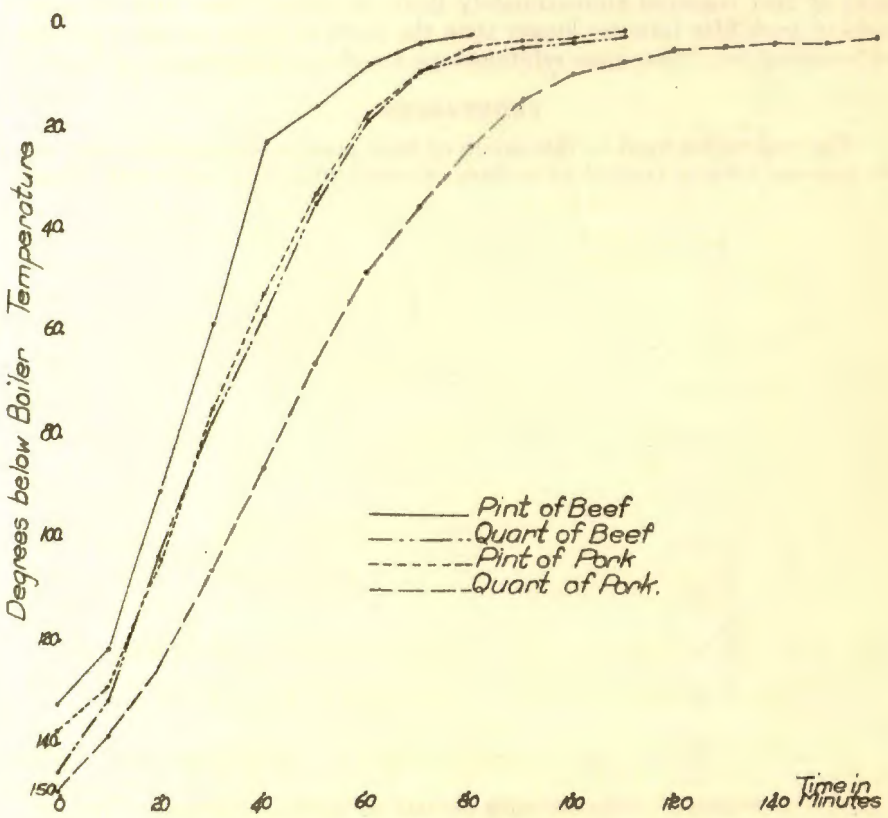

Figure 3. Comparison of heat penetration curves for quart and pint packs of beef and pork in hot water bath.

the jar had been filled with water. With the suet solid, the heating was entirely by conduction, but when the suet became liquefied the heating was

largely by convection. Thus beef fat seems to retard heat penetration as long as it is in a solid condition, but allows for more rapid heat penetration when it is melted.

Pork. The variables used in the packs of pork were: pork loin with and without water, pork sausage plain and with added fat, and pork sausage made into cakes and precooked.

When the heat penetration for the beef and pork packs was compared, it was noted that the jars of pork heated more slowly as a rule than the beef. The heat penetration with the various packs of pork was so nearly the same that the differences in time noted were not significant. The line shown for pork in figure 3 is representative of the rate at which the pork heated in the various packs.

Pint versus quart packs of beef and pork. Comparing the time interval for reaching processing temperature in pint versus quart packs of beef and pork in the boiling water bath it was observed that the quart packs of beef required approximately thirty minutes longer and the quart packs of pork fifty minutes longer than the pints of either to reach processing temperature. These time relationships are shown graphically in figure 3.

VEGETABLES

The vegetables used in this series of heat penetration studies were chosen because each is typical of a class of vegetables, and the result should

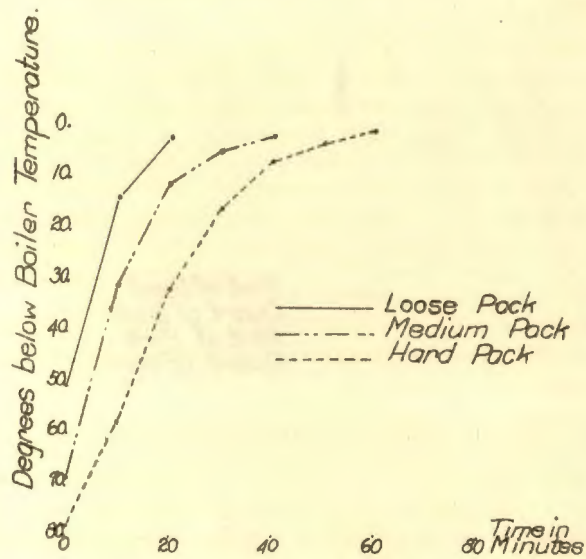

Figure 4. Effect of pack on rate of heating of green beans in hot water bath.

apply to all other vegetables in the same class. Green beans, asparagus, spinach, tomatoes, and sweet corn were used in the experiments and the results of each will be discussed under the individual headings.

Green beans—Three different packs were used for the green beans: 212, 340, and 425 grams of beans per pint. The more compact the pack of beans, the longer the time required for the inner temperature of the jar to reach the temperature of the water bath or pressure cooker.

The loose pack in the pint glass jar reached processing temperature in twenty minutes time, the medium pack reached it in forty minutes time, and the hard pack required sixty minutes. (See Figure 4.) This difference in time required to reach processing should be taken into account when formulating tables for processing green beans. It is probable that a good time table should specify the most desirable weights of beans to be included in the different sized jars as well as the length of processing time for each.

The tightness of the hard pack in these experiments with beans was such that the appearance of the beans was somewhat impaired. In order to conserve the appearance of the vegetable a slightly looser pack would be more desirable. A consideration other than the appearance of the finished product is the added time required for the contents of a tightly packed jar to reach retort temperature—forty minutes more being required in this experiment for the beans in the hard pack than for those in the loose pack. This may partially account for many of the failures in the home canning of beans.

The results of the experiments with beans in the pressure cooker are similar to those given above for the water bath. The differences in time required for the different packs to approach retort temperature in the pressure cooker were 20, 30, and 60 minutes, respectively, after the closing of the petcock.

Spinach—The variations in the packs of spinach used were 204, 375, and 475 grams of spinach per pint. These packs were practically the same as those used by Castle (12). With spinach as with beans the looser the pack, the more quickly the spinach reached the processing temperature.

The loose pack of spinach as used in these experiments was not practical due to the very large amount of water, one and one-half cups per pint, which had to be added to fill the jars. The medium pack, 375 grams per pint, would ordinarily be considered a loose pack. During the processing period, the spinach tended to pack together and draw away from the sides of the jar, leaving free liquid near the outside of the jar.

Figure 5 shows the heat penetration in the loose, medium, and hard packs of spinach with the temperature of the retort during processing in the pressure cooker.

Asparagus—Only two different packs of asparagus were used, 260 and 320 grams of asparagus per pint. The packs would be considered as loose and medium. The effect of using the tips of asparagus cut in one inch pieces was tried. The differences in time for reaching bath or pressure cooker temperature for the two different packs were so slight as to be practically of no consequence.

An additional experiment which indicated the effect of initial temperature on rate of rise of temperature was tried. The conditions in this test were not the same as those in the others in that the water bath temperature was not recorded, nor were the jars heated until constant in temperature, but only until all three jars showed the same temperature at the center. Asparagus in all three cases was cut into one inch pieces and 300 grams

used per pint. The contents of the first jar were precooked, of the second scalded, and of the third packed into the jar cold and covered with boiling water. The initial temperatures in the three jars were 185° F., 182° F., and 133° F., respectively. At the end of 20 minutes heating in the water bath, all three jars had reached a temperature of 205° F. This is in accordance with the results of Magoon and Culpepper (22), who show that two jars with different initial temperatures in the same retort reach retort temperature at practically the same time. Thus the jar with the lower

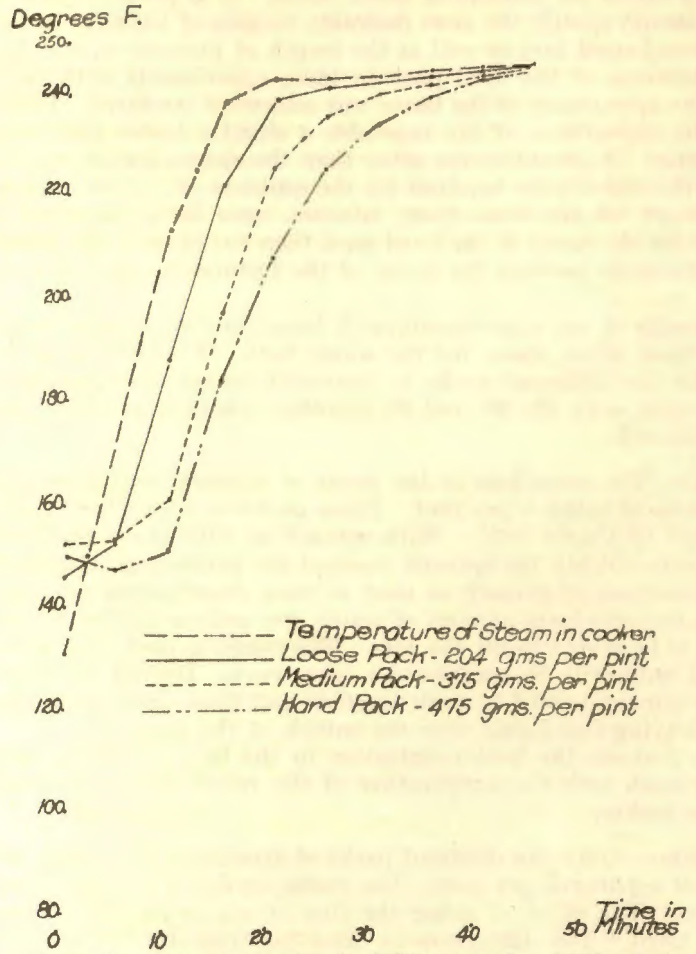

Figure 5. Heat penetration in different packs of spinach in the pressure cooker.

initial temperature heats at a faster rate than the one with the higher initial temperature.

This would also apply to vegetables such as beans and peas, which are like asparagus, in that the pack allows free liquid to fill the interspaces and thus convection currents are quickly set up.

Tomatoes—The tomatoes were processed as tomato pulp and puree. The pack used was 500 grams of tomato per pint and 950 grams per quart. A final temperature as high as that of the water bath or pressure cooker is not necessary in canning tomatoes. The sterilizing temperature for tomatoes need not be as high as for other vegetables, because of their natural acidity. Consequently a much shorter processing time is required than for other vegetables. The tomatoes were processed in the water bath for 5, 12, 20, 25, 35, 45 minutes and in one instance until constant in temperature, i.e. 70 minutes. In the pressure cooker they were processed at 5 lbs. pressure for 20 minutes, 10 lbs. pressure for 10 and 15 minutes and at 15 lbs. pressure for 10 minutes.

When the tomatoes were removed from the water bath or the gas turned off under the pressure cooker, the temperature in the center of the jars continued to rise. Since the temperature at the center of the jar was not as high as that near the outside, the heat radiated both into and out of the jar. The lower the temperature at the center of the jar at the end of the processing period the longer the interior tmperature continued to rise, with the exception of the five minute process in which the temperature

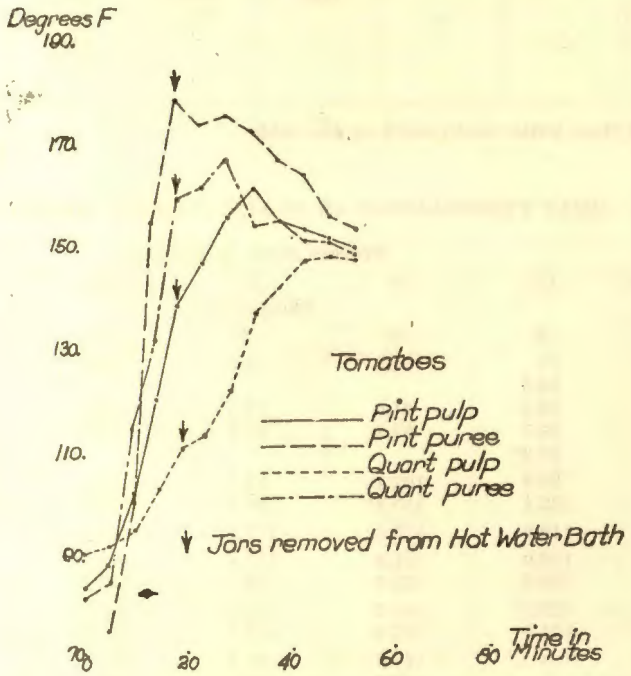

Figure 6. Heat penetration in tomato pulp and puree, pints and quarts, in the hot water bath.

continued to rise for five minutes less time than in the 12 minute process. This was probably due to the fact that the temperature of the tomato near the outside of the jar rose only a few degrees in the short processing period. The interior temperature of the tomatoes heated to a constant temperature

TABLE I. HEAT PENETRATION IN PINT JARS OF TOMATO PULP.

	Process time in minutes					
	5	12	20	25	35	45
Elapse of time			Temperature			
Minutes	°F	°F	°F	°F	°F	°F
0	84.6	70.0	84.0	74.2	76.2	88.5
3		72.3				
5	88.8*	74.6	88.6	78.6	79.3	93.1
10	89.6	85.6	101.4	84.5	82.4	104.0
12		89.2*				
15	104.6	92.8	120.0	105.5	104.4	123.2
20	114.7	114.8	138.5*	124.1	126.4	141.0
25	117.8	126.8	146.6	143.5*	141.8	158.1
30	119.6	128.9	155.5	150.0	157.2	170.8
35	118.5	131.8	160.5	161.6	173.0*	182.6
40	116.2	135.0	154.5	162.2	173.8	190.4
45			152.7	162.7	181.0	194.8*
50			150.5	160.0	183.6	189.1
55			148.1	156.6	168.3	190.0
60				152.5	154.2	175.2
65					141.6	160.3
70					137.6	147.5
75						140.1

*Jars removed from boiler and placed on the table.

TABLE II. HEAT PENETRATION IN QUART JARS OF TOMATO PULP.

	Process time in minutes				
Elapse of time	12	20	25	35	45
	Temperature				
Minutes	°F	°F	°F	°F	°F
0	86	90.5	73.0	84.7	81.1
3	88.5				
5	85.8	91.6	74.4	84.7	81.7
10	85.7	94.8	75.7	87.5	84.7
12	87.9*				
15	90.6	102.4	82.4	97.8	93.3
20	102.8	110.4*	90.1	107.4	104.0
25	110.9	113.0	112.2*	119.3	115.0
30	116.0	121.8	111.4	132.0	127.1
35	120.5	135.6	128.2	143.1*	139.8
40	123.2	141.3	137.2	146.8	147.8
45	124.4	146.9	142.4	155.2	156.8*
50		147.2	145.4	161.6	156.2
55		146.3	145.2	167.7	167.0
60			145.0	163.4	167.9
65				160.7	162.7
70				157.2	159.7
75					153.7

*Jars removed from boiler and placed on table.

did not continue to rise after being removed from the water bath, due to the fact that the temperature at the center of the jar was as high as that near the outside on removal.

The tomato puree, which was of uniform consistency, heated more quickly than the tomato pulp. This was true for both pints and quarts, with the quart of tomato puree heating faster than the pint of tomato pulp. This is illustrated in figure 6.

The tomatoes on removal from the bath in the 20 and 25 minute process were at a temperature of approximately 140° F. when removed. Because of the continued rise in temperature following removal from the bath it will be noted that the tomatoes were at a temperature above 140° F. for

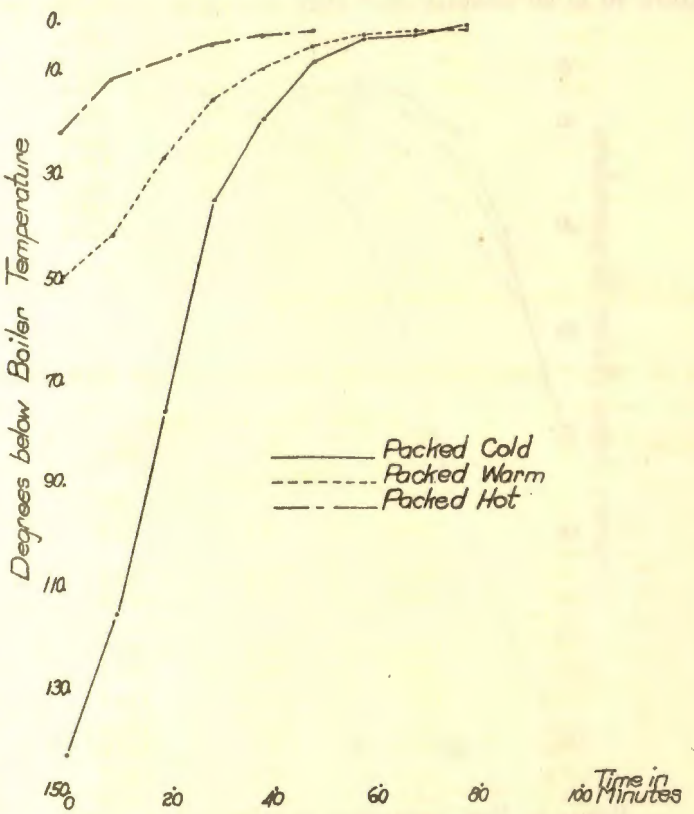

Figure 7. Heat penetration in sweet corn packed at different initial temperatures.

a period of 35 minutes. A part of the data for the boiling water bath processed tomatoes is given in tables I and II.

Sweet corn. The sweet corn used in these experiments was mature Stowell's Evergreen. It was cut from the cob "Maine style", the tips being cut away with a sharp knife and the milk scraped from the cob.

Three different packs were used for the sweet corn, 274, 320 and 384 grams per pint. The water added was 206, 160, and 96 grams, respectively. When pint jars containing these varying amounts of corn were heated in the boiling water bath the loose pack reached processing temperature in 50 minutes, and the medium and solid packs in 90 minutes. Quart jars with a loose pack reached processing temperature 60 minutes sooner than the quarts with a medium or solid pack. In the pressure cooker, pint and quart jars of corn approached retort temperature 20 minutes sooner in the loose pack than in the medium and solid packs. From the standpoint of heat penetration the more water that is added to the corn the sooner the desired temperature is reached.

In the hot water bath, quart jars of sweet corn reached processing temperature 40 to 60 minutes later than pint jars under the same condi-

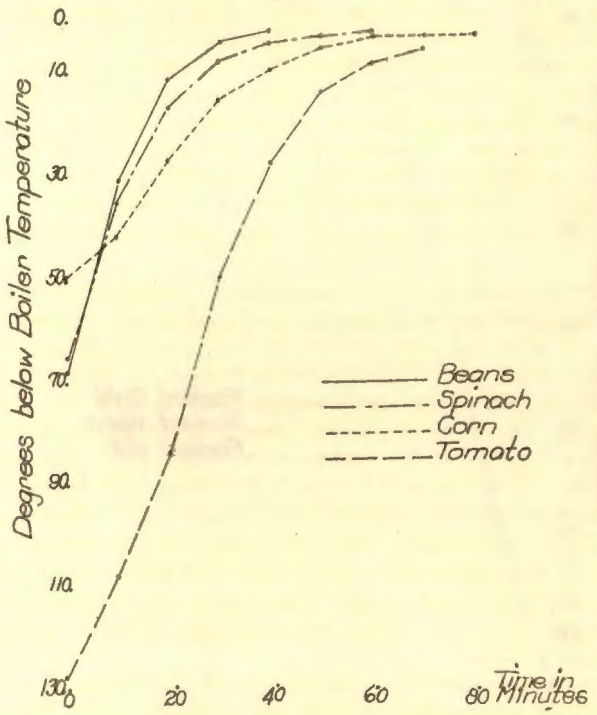

Figure 8. Heat penetration as influenced by consistency of vegetables.

tions. In the pressure cooker, the difference between the pints and quarts was about 20 minutes.

The initial temperature of the sweet corn at time of packing was important in determining the length of time required for the corn to reach processing temperature. Figure 7 shows comparative curves for medium packs of sweet corn packed at different initial temperatures. It will be noted that the corn packed hot reached processing temperature 30 min-

utes sooner than that packed warm or cold. This advantage in time was not observed in jars processed under pressure, as in this case the jars with different packs reached 212° F. at practically the same time. However, according to Harrison (19) a 2° F. increase in the initial temperature in the range of 180° to 200° F. may be considered as equivalent to adding one minute to the processing time. (Processed at 245°F.) He stated that 20 minutes should be added to the processing period if the center of the can is 122° instead of 182° F. (Processed at 252° F.)

Heat penetration in corn is slow because of the pasty or viscous consistency, thus any factors which decrease the time required for the center of the jar to reach maximum temperature should be observed. The use of a loose pack and a high initial temperature in the home canning of corn should have a favorable influence on the keeping qualities.

Consistency of the vegetable. To illustrate the influence which the composition of the material has upon the rate of heat penetration, curves depicting the heat penetration in medium pint packs of beans, spinach, corn and tomato are compared in figure 8. A vegetable of the consistency of corn would be expected to take a longer time to reach processing temperature because of its more viscous composition. The corn took 40 minutes longer than the beans. The time for the spinach was intermediary between that for corn and beans. The rate of penetration in tomato pulp is similar to that of corn. The tomatoes started at a lower temperature and were removed before the contents of the can reached the temperature of the water bath.

Pint versus quart packs of vegetables. Medium packs of corn, beans, asparagus, spinach and tomatoes in pint and in quart jars were compared for length of time required to reach the processing temperature in the boiling water bath. It took one hour longer to heat the medium quart pack of corn to processing temperature than it took for the medium pint pack of corn. In the case of the beans, asparagus and spinach, it took 10-20 minutes longer for the quarts of medium pack than for the pints of medium pack. The tomatoes in quart jars took approximately 10 minutes longer to reach an effective processing temperature in the centers of the jars.

Special pressure cooker tests—In order to determine the best method for the use of the petcock for maximum efficiency in the operation of the pressure cooker*, a series of tests were made leaving the petcock open for varying lengths of time. Green beans in the different packs were used in the first series of tests.

The pressure recorded on the gauge of the pressure cooker is used as an index of the temperature inside the cooker. With air left inside the cooker, however, a pressure may be obtained which causes the gauge to indicate a certain temperature when in reality the temperature is not as high as the pressure indicates.

Ball (3) states that the uniformity of temperature within the retort depends largely upon whether or not there is air in the retort and that in pure steam processing, venting should be sufficient to remove the air quickly after the steam is turned on.

The Anchor Cap and Closure Corporation (1) report with regard to retort control that in using a retort which has the vents closed throughout

*A National Pressure Cooker No. 25 was used.

the entire process, thus necessitating higher pressures to secure the desired temperature, the pressure developed inside the sealed retort will equalize the pressure developed inside the glass containers.

Harrison (19) secured a temperature of 250° F. in four minutes with both the safety valve and the bleeder valves of a commercial retort open, but it took 15 minutes to secure the same temperature with only the bleeder valves open.

The following technique was used for the pressure cooker experiments. The petcock was either kept closed throughout, or closed with the appearance of steam or left open for varying lengths of time after the appearance of steam, 2, 3, 5, and 7 minute intervals being tried.

The theoretical temperatures on the basis of the pressure gauge readings were compared with the actual temperatures observed by thermocouple determinations. These are recorded in table III.

TABLE III. PRESSURE COOKER AND AUTOCLAVE OPERATION TESTS.

Test 1—Pressure Cooker

Fet Cock Closed	Observed Pressure Gauge Readings (Average)	Temp. on basis of Gauge Readings	Observed Temp. of Steam in Retort (Average)	Difference between temps. indicated by pressure gauge and observed reading
	lbs.	°F	°F	°F
Throughout	12	243	193.5	49.5
At appear. of steam	13.6	246	231.2	14.1
3 min. after steam	13.2	246	238	8
7 min. after steam	15	249	244	5

Test 2—Pressure Cooker

Petcock closed				
	lbs.	°F	°F	°F
Throughout	15	249	210	39
At appear. of steam	15	249	232	17
3 min. after steam	15	249	241	8
5 min. after steam	15	249	245	4
7 min. after steam	15	249	245	4

Test 3—Autoclave

Petcock closed				
	lbs.	°F	°F	°F
Throughout	15	249	197.6	51.4
At appear. of steam	15	249	203.0	46.0
2 min. after steam	15	249	222.8	26.1
5 min. after steam	15	249	242.6	6.4
7 min. after steam	15	249	244.4	4.6

It will be observed that when the petcock was not closed until steam had escaped for 7 to 10 minutes after the first appearance of steam, that with the gauge reading of 15 lbs. the difference between the observed and theoretical temperatures of the steam was 5° F. The differences observed in the other tests increase as the time for allowing enclosed air to escape

is shortened. The maximum difference between theoretical and observed readings was obtained with the petcock kept closed throughout the test.

A second test was conducted with the pressure cooker keeping the gauge readings uniformly at 15 lbs. pressure throughout and with water in the pint jars instead of beans. The differences between retort and theoretical readings are similar to those obtained in the first tests. See table III and figure 9 for presentation of results obtained in second test.

A third test was conducted using an autoclave* (30 by 20 inches) instead of the pressure cooker. The steam was generated by a high pressure steam coil. The differences in temperatures between autoclave temperature and theoretical temperatures based on pressure gauge readings are even more pronounced in this series. See table III.

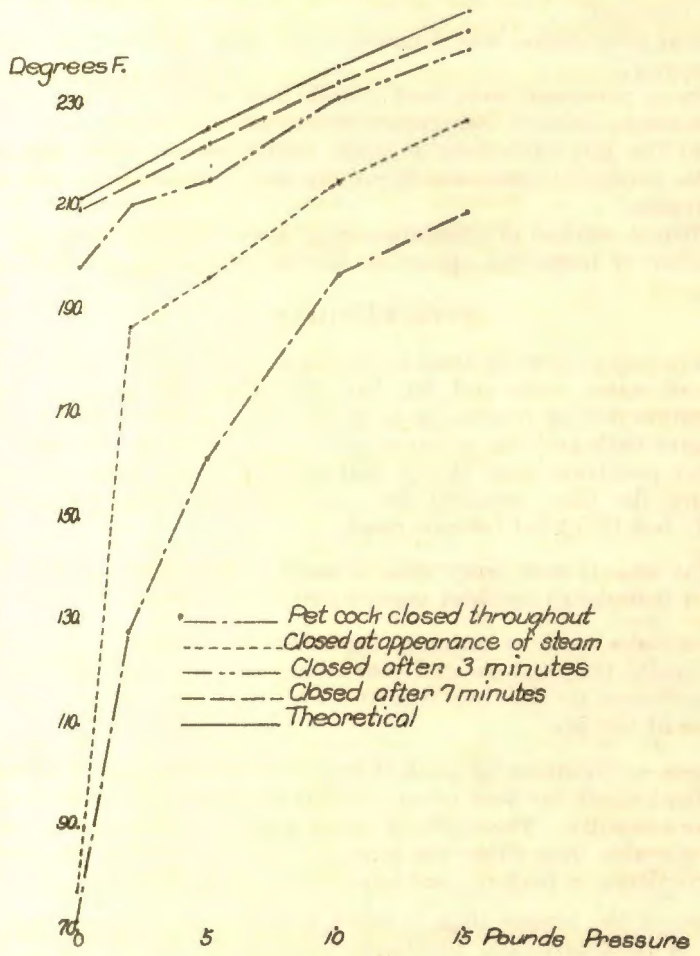

Figure 9. Variation in interior temperature of pressure cooker due to petcock technique used.

*No. 1686. Horizontal Autoclave. Arthur H. Thomas Co., Philadelphia.

From the tests described, it is evident that marked differences in retort temperature can be obtained, depending on the technique of operating the pressure cooker. The differences in temprature are great enough to account for spoilage in products canned in the pressure cooker if inefficient technique is used. Bigelow (8) emphasizes the importance of accurate temperature, noting that in processing peas if 239° F. instead of 240° F. was used an additional six minutes should be added to the processing period.

SUMMARY

Experiments on the heat penetration into pint and quart glass jars of vegetables and meats processed in the hot water bath and pressure cooker are reported.

The heat penetration was determined by means of thermocouples and a potentiometer.

The foods processed were beef round, beef suet, pork loin, pork sausage, green beans, spinach, asparagus, sweet corn and tomatoes.

Size of the jar, variations in pack, initial temperature, and consistency of the products were considered for their influence on the time of heat penetration.

An efficient method of pressure cooker operation was determined, and the possibility of inefficient operation during processing pointed out.

CONCLUSIONS

1. The variables ordinarily used in packing canned meat, such as the addition of water, bone and fat, have but slight influence on the heat penetration during processing in glass. This is true both for the boiling water bath and the pressure cooker. Precooking the meats makes the heat penetrate more slowly, but because of the higher initial temperature the time required for reaching processing temperature is slightly less than that for raw meat.

2. Beef fat retards heat penetration in meat as long as it is solid, whereas when it is liquefied the heat penetration is very rapid.

3. The variables used in canning vegetables, as size of jar, closeness of pack, initial temperature and consistency of the vegetable, have a decided influence on the time required for penetration of the heat to the interior of the jar.

4. Closeness or tightness of pack delays heat penetration by influencing the rate at which the heat penetrates the product—the looser pack heating more rapidly. The medium packs used in these experiments were more desirable than either the loose or hard packs from the standpoint of convenience in packing and appearance of the product.

5. Because of the longer time it takes for the heat to penetrate to the center of the quart jars, additional processing time should be allowed when using time tables specified for pints. Additional time lengths suggested are; for beans, asparagus and spinach 10-20 minutes, for tomatoes 10 minutes, for sweet corn 60 minutes, for beef 30 minutes, and for pork 50 minutes.

6. Unless precautions are taken to allow the exhaustion of the air in the pressure cooker before closing the petcock, a lower temperature is obtained than that indicated by the pressure gauge.

7. Leaving the petcock of the pressure cooker open for seven minutes after the appearance of steam gave the maximum temperature under the conditions of the experiments.

LITERATURE CITED

1. ANON.
1927. A distinct advance is made in the technique of processing glass packed foods. Canning Age, 8:124-128.

2. BALL, C. O.
1923. Thermal process time for canned food. National Research Council Bull. No. 7, Pt. 1.

3. BALL, C. O.
1927. Theory and practice in processing. The Canner, 64, No. 5, 27-32.

4. BAUER, V.
1923. Temperature inside the jar during cold pack canning. Jour. Home Econ. 15, 381-382.

5. BELSER, JOSEPH.
1905. Studien Uber Verdorhene Gemusekonserven. Arch. Hyg. Bd. 54, Heft. 2, 107.

6. BEVERIDGE, W. W. O. AND FAWCUS, H. B.
1908. Experiments with preserved meat. Royal Army Medical Corps. Jour. 10, 315.

7. BIDAULT.
1923. Penetration of heat in canned foods during sterilization. Bull. Soc. Hyg. Aliment. 10, 471-486. Chem. Abs. 17, 1088.

8. BIGELOW, W. D.
1922. Some precautions in canning process. II. Dangers to be avoided in pea canning. Amer. Food Jour. 17, 11-12 (April).

9. BIGELOW, W. D., BOHART, G. S., RICHARDSON, A. C., BALL, C. O.
1920. Heat penetration in processing canned foods. Bull. Res. Lab. Nat'l. Canners' Assn. No. 16-L, 1-128.

10. BITTING, A. W.
1925. Heat penetration in glass jars, especially in home canning. The Canning Trade, 48, 8.

11. BOVIE, W. T., AND BRONFENBRENNER, J.
1919. Studies on canning—An apparatus for measuring the rate of heat penetration. Jour. Ind. & Eng. Chem. 11, 568.

12. CASTLE, CARRIE E.
1919. Effect of pack and depth of water bath upon the interior temperature of jars in cold pack canning. Jour. Home Econ. 11, 246-252.

13. DENTON, M. O.
1918. What temperature is reached inside the jar during home canning. Jour. Home Econ. 10, 548-552.

14 DUGDALE, CLARICE M.
1924. The preservation of food by sterilization. Jour. Soc. Chem. Ind. 43, 17-23.

15. DUGDALE, CLARICE M.
1924. The handling of chemical and bacteriological problems in preserving food. State Med. 31, 522-528, 1923. Chem. Abs. 18, 555.

CATALASE AND OXIDASE OF THE TOMATO AS INFLUENCED BY THE SOIL REACTION*

E. S. HABER

From the Department of Horticulture, Iowa State College.

Accepted for publication September 20, 1928.

One of the important alterations occurring in bench soils in greenhouses is the change from a slightly acid reaction, common to most soils when first placed in the benches, to a highly alkaline reaction, due to the addition of alkaline material in watering.

The present problem was undertaken to determine the effect of the soil reaction (pH value), first, on the growth of the tomato plant, and, second, its effect on catalase and oxidase activity.

No attempt will be made to give a complete review of the literature pertaining to catalase and oxidase activity. Only those references will be cited which have a direct relation to the data given here, since Ezell and Crist (7), Heinicke (10), Knott (13), Rhine (16) and others have covered the subject thoroughly.

Ezell and Crist (7), working with lettuce, radish and spinach plants, found only a slight negative correlation between oxidase activity and growth or size of the plants, but the catalase activity of the same plants was negatively significant. Reed (15) demonstrated that oxidase and catalase were independent of each other and that in the ripening of fruit catalase increased while oxidase remained constant or nearly so.

Catalase activity in relation to growth in fruit trees, especially the apple, has been studied considerably by Heinicke and his co-workers. Heinicke (10) found growth-producing substances increased catalase activity while substances which tended to inhibit vegetative activity had a retarding influence on catalase activity. Organic nitrogenous materials seemed to increase the activity while carbohydrates were believed to be the chief cause of reduction in catalase activity. The same author (11) found apple trees grown on sandy soil showed less catalase activity in the leaves than those from trees growing on a clay soil, whether the trees were cultivated or grown on sod. Apple trees when grown in sod and given applications of nitrate of soda up to 8 ounces per tree showed an increase in catalase activity of the leaves. Heinicke (12) also determined that fruiting tends to reduce catalase activity in the bark of the apple tree. By applying nitrates to only one side of the trees Auchter (3) obtained increased catalase activity on the nitrated side. Biechy (5) found that an addition of potassium fertilizer decreased the catalase activity of the plant. According to Knott (13) the catalase activity of spinach leaves was not influenced either by vegetative or reproductive type of growth.

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*Part II of a thesis submitted to the Graduate Faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Grüss (8) reported that it was impossible to make quantitative determinations of catalase in fresh potato extract on account of the rapid degeneration during and after grinding. Appleman (2), however, discovered that if the potato was ground with calcium carbonate to neutralize the acids freed by the grinding, diluted immediately and kept at 20° C. or below, this rapid degeneration was overcome and comparable results could be secured without any difficulty. Heinicke (10) found that the amount of calcium carbonate equal to the green weight of the tissue was in excess of that needed for the acidity to be corrected, but far more could be added without affecting the reaction. Becking and Hampton (4) using sodium carbonate to neutralize the plant acids concurred in this belief. According to Knott (14) catalase activity of the tomato and spinach decreased more slowly at cool temperatures.

According to Ezell and Crist (7) samples of tissue prepared for the determination of oxidase activity should be allowed to stand for about six hours before using. Oxidase activity of the sample increased for about six hours and then remained constant for ten or twelve hours, after which there was a slow decline.

MATERIALS AND METHODS

New compost soil was placed in the greenhouse bench. The individual plots were separated by boards that extended the entire depth of the bench in order to prevent the soil of one plot from mixing with that of another. The reaction (pH value) of the soil when placed in the benches was 6.5.

Since the soil was always acid when placed in the bench and became highly alkaline due to the addative effect of salts in watering, three reactions (pH values) were decided upon, one extremely alkaline, pH8.5-9.0, one neutral or nearly so, pH 6.5-7.0, and one extremely acid, pH 4.0-4.5. These plots were all run in duplicate. To secure the alkaline reaction, the soil was treated with hydrated lime in sufficient quantity so that the pH value was about 9.0 a week after treatment, when a fair degree of equilibrium was reached. For the neutral plots the soil was not treated, since it was nearly neutral without treatment. The acid reaction was secured by adding phosphoric acid (H_3PO_4) so that the reaction was 4.0 a week after treatment.

The tomato plants used were of the Bonny Best variety. The seed was sown in a flat, the seedlings pricked off when one and one-half inches high and planted in two-inch pots, later shifted to four-inch pots and finally transferred to the treated plots ten days after the soil had been treated The soil used in potting was ordinary compost with a pH value of 6.0-6.5.

The fall crop was placed in the greenhouse bench October 1 and completed its growth February 1. The spring crop was placed in the bench February 20. Determinations of the pH values of the soil of the various plots were made at ten-day intervals after the crop was benched. Small amounts of hydrated lime or phosphoric acid were added from time to time to keep the pH within the desired range.

To secure the desired information regarding growth and fruit production, yields were recorded by weighing the fruits when ripe. Relative growth on the various plots was secured after the fruit had been harvested. The plants were dug, the roots washed free of soil with tap water and then washed with distilled water. Roots, stems and leaves were separated

from each plant and air-dried in the laboratory for four weeks with the temperature ranging from 75° to 85° C., and then weighed to determine the relative amount of growth. Catalase and oxidase activity were measured on fresh material of leaves and fruit on both spring and fall crop.

For measurement of catalase activity essentially the same method was employed for preparation of samples as that used by Ezell and Crist (7). A composite sample from several plants was made by means of a Ganong leaf punch. The leaves from which samples were taken were just reaching vegetative maturity and samples were taken from the same aged material each time to secure comparable results, since young tissue had been found by numerous investigators to be more active with regard to most enzymes than old tissue. One gram of leaf tissue weighed immediately after removal from the plant was used for all leaf determinations. Samples of the fruits were taken by punching out a cylinder by means of a cork borer one centimeter in diameter. Fruits of approximately the same size and degree of maturity were used and the sample weighed immediately. The sample of both leaf and fruit was mixed with an equal weight of dry calcium carbonate, then 2 c.c. of distilled water added and the mixture ground gently in a mortar until a uniform creamy mixture was obtained. Distilled water was then added so that the fresh ground tissue was suspended in 25 c.c. of water. The solution was then placed in a tightly stoppered bottle and kept on ice until used.

Catalase activity was determined by Harvey's (9) modification of the Bunzel oxidase apparatus. Two c.c. of the plant solution were placed in the short arm of the tube and 5 c.c. of hydrogen peroxide in the long arm of the tube. The tube was suspended in a DeKhotinsky water bath held constant at 35° C. Uniform shaking was accomplished by means of a motor-driven mechanical shaker. The oscillations were timed so as to cause the solution to flow from one end of the tube to the other at the rate of 90 times per minute. Before shaking commenced the tube containing the materials was placed in the bath and allowed to stand for ten minutes until it had reached the temperature of the bath. The tube was then shaken for three minutes and the amount of oxygen evolved measured.

For the measurements of oxidase activity, portions of samples prepared for catalase determinations were used for oxidase determinations. The procedure was essentially the same as that used by Ezell and Crist (7). Two c.c. of the prepared sample were placed in the short arm of the tube, while 5 c.c. of a fresh one percent pyrogallol solution were placed in the long arm of the tube. An alkali vial containing 1 c.c. of normal sodium hydroxide was put in place and the manometer adjusted. The reaction tube was placed in the bath 10 minutes before shaking to allow the materials to reach the temperature of the bath. The reading of the manometer at the end of 1 hour was taken as a measure of oxidase activity.

Checks were run on all samples for both catalase and oxidase. All results given are from samples which checked within 0.2 c.c. Samples from the same plot taken the same day checked within this range. All results given are averages from four samples.

EXPERIMENTAL WORK

EFFECT OF SOIL REACTION ON GROWTH AND YIELD

As will be noted in table I the soil reaction had considerable effect on both yield and growth of the tomato.

TABLE I. EFFECT OF SOIL REACTION ON GROWTH AND YIELD OF TOMATO PLANTS.

Soil Reaction	Yield per plant	Average weight per plant Material air-dried			
		Roots	Stems	Leaves	Total
pH 8.5-9.0	2.52 lbs.	2.52 gms.	32.90 gms.	56.90 gms.	92.32
pH 6.5-7.0	4.06 lbs.	2.30 gms.	31.25 gms.	79.80 gms.	113.35
pH 4.0-4.5	3.04 lbs.	2.34 gms.	24.74 gms.	57.84 gms.	84.92

Two plants on one of the acid plots and three plants on the alkaline plots showed evidence of mosaic, and were discarded. One of the vines on an acid plot in the series became infected with wilt and was likewise discarded.

The largest yield per plant was secured on plots where the soil reaction had a pH value of 6.5-7.0 and the smallest yield on the alkaline plots, pH 8.5-9.0. Maximum root growth as measured by the weight of the air-dried material occurred on the alkaline plots. There was very little difference in root growth between plants grown on the neutral or acid plots. The greatest amount of total dry matter was secured on the plots with a neutral soil reaction, while the alkaline plots produced only 81.4 percent as much total dry matter as the neutral plots, and the acid plot only 74.9 percent as much.

EFFECT ON CATALASE AND OXIDASE ACTIVITY

Measurement of catalase and oxidase activity were made on the leaves and fruit of both fall and spring crop. Tables II to V, inclusive, give the results of the findings with respect to oxidase and catalase activity for the fall crop.

Differences in catalase activity were most pronounced in the case of the green mature fruits. Fruits on plants from soils with a reaction of pH 6.5-7.0 consistently showed less catalase activity than those from soils with pH 8.5-9.0 and 4.0-4.5.

Fully ripe fruits from the neutral plots also showed less activity than those from the acid or alkaline plots, but the differences were not so marked as in the green mature fruit. No consistent differences in catalase activity were noted in the very green fruits, but catalase activity was at the minimum at this stage of maturity, so if there were any differences, they were not significant.

TABLE II. CATALASE AND OXIDASE ACTIVITY OF TOMATO FRUITS OF DIFFERENT STAGES OF MATURITY GROWN IN SOILS WITH DIFFERENT pH VALUES.

December 20

Soil Reaction	Oxidase c.c. of Hg displaced at end of 60 minutes	Catalase (c.c. of water displaced) Time in minutes		
		1	2	3
Green Mature Fruit				
pH 8.5-9.0	1.70	1.1	1.3	1.5
pH 6.5-7.0	1.50	0.8	0.9	0.9
pH 4.0-4.5	1.80	2.7	3.3	3.7
Ripe Fruit				
pH 8.5-9.0	1.65	0.9	1.1	1.3
pH 6.5-7.0	1.75	1.3	1.5	1.7
pH 4.0-4.5	1.35	1.4	1.7	1.9
Green Fruit				
pH 8.5-9.0	1.10	0.6	0.7	0.7
pH 6.5-7.0	1.25	0.7	0.9	1.0
pH 4.0-4.5	1.30	0.7	0.8	0.9

TABLE III. CATALASE AND OXIDASE ACTIVITY OF TOMATO FRUITS OF DIFFERENT STAGES OF MATURITY GROWN IN SOILS WITH DIFFERENT pH VALUES.

January 5

Soil Reaction	Oxidase c.c. of Hg displaced at end of 60 minutes	Catalase (c.c. of water displaced) Time in minutes		
		1	2	3
Green Mature Fruit				
pH 8.5-9.0	1.40	1.6	1.8	2.0
pH 6.5-7.0	1.55	1.0	1.2	1.3
pH 4.0-4.5	1.70	2.7	3.0	3.3
Ripe Fruit				
pH 8.5-9.0	2.00	2.3	2.7	2.9
pH 6.5-7.0	1.95	0.8	1.0	1.1
pH 4.0-4.5	2.10	0.9	1.1	1.2
Very Green Fruit				
pH 8.5-9.0	.95	0.5	0.7	0.8
pH 6.5-7.0	1.05	0.5	0.6	0.6
pH 4.0-4.5	1.25	0.7	0.9	1.0

TABLE IV. CATALASE AND OXIDASE ATCIVITY OF TOMATO FRUITS OF DIFFERENT STAGES OF MATURITY GROWN IN SOILS WITH DIFFERENT pH VALUES

January 19

Soil Reaction	Oxidase c.c. of Hg displaced at end of 60 minutes	Catalase (c.c. of water displaced) Time in minutes		
		1	2	3
Green Mature Fruit				
pH 8.5-9.0	1.80	2.2	2.4	2.6
pH 6.5-7.0	1.75	1.0	1.2	1.3
pH 4.0-4.5	1.90	2.4	2.7	2.9
Ripe Fruit				
pH 8.5-9.0	2.10	0.9	1.0	1.1
pH 6.5-7.0	2.18	0.9	1.0	1.1
pH 4.0-4.5	1.90	1.0	1.3	1.4
Green Fruit				
pH 8.5-9.0	1.00	0.7	0.9	1.0
pH 6.5-9.0	.90	0.7	0.8	0.9
pH 4.0-4.5	.90	0.6	0.7	0.7

TABLE V. CATALASE AND OXIDASE ACTIVITY OF TOMATO FRUITS OF DIFFERENT STAGES OF MATURITY GROWN IN SOILS WITH DIFFERENT pH VALUES.

February 1

Soil Reaction	Oxidase c.c. of Hg displaced at end of 60 minutes	Catalase (c.c. of water displaced) Time in minutes		
		1	2	3
Green Mature Fruit				
pH 8.5-9.0	1.90	2.0	2.2	2.4
pH 6.5-7.0	1.90	1.2	1.3	1.3
pH 4.0-4.5	1.80	2.4	2.7	2.9
Ripe Fruit				
pH 8.5-9.0	1.75	1.0	1.3	1.5
pH 6.5-7.0	1.90	1.3	1.5	1.6
pH 4.0-4.5	2.00	1.4	1.7	1.9
Green Fruit				
pH 8.5-9.0	1.10	0.6	0.7	0.7
pH 6.5-7.0	1.15	0.7	0.8	0.9
pH 4.0-4.5	1.25	0.7	0.9	1.0

Oxidase activity was greater in ripe than in the very green or green mature stages, but this was independent of the soil reaction or growth and yield. The oxidase activity of ripe fruits from acid, alkaline or neutral plots was practically the same.

Since the least catalase activity occurred in fruits taken from plots of pH 6.5-7.0, it was thought that this difference in activity might be due to differences in pH value of the fruit in various stages of ripening. The juice of the fruits was squeezed out through several layers of cheese cloth and the pH value of the juice determined by the quinhydrone method. The following table shows the results.

TABLE VI. THE pH VALUE OF THE TOMATO FRUIT IN DIFFERENT STAGES OF RIPENING.

Soil Reaction	Condition of fruit	pH January 13	pH January 20
pH 8.5-9.0	Ripe	4.25	4.11
	Mature Green	4.03	3.91
	Green	4.11	3.98
pH 6.5-7.0	Ripe	4.25	4.11
	Mature Green	3.75	3.94
	Green	3.96	4.08
pH 4.0-4.5	Ripe	4.29	4.13
	Mature Green	4.04	3.95
	Green	4.06	4.03

In general, the changes in pH value were not great, but this might be expected in a well-buffered solution such as that of the fruit juice. In ripe, mature green and very green samples taken from the same plot the pH value was slightly lower in the green mature stage than in the very green or ripe stages. However, the differences were not great enough to account for the increase or decrease in catalase activity at any particular stage of development of the fruit or from any particular soil reaction.

Catalase and oxidase activity in the leaves was measured first on December 20 when the fruits were beginning to ripen; again on January 12 when about one-half of the crop had been picked and again on February 1 when practically all of the crop had matured. (Table VII.)

TABLE VII. CATALASE AND OXIDASE ACTIVITY OF TOMATO LEAVES GROWN IN SOILS WITH DIFFERENT pH VALUES.

Soil Reaction	Oxidase c.c. of Hg displaced at end of 60 minutes	Catalase (c.c. of water displaced) Time in minutes		
		1	2	3
December 20				
pH 8.5-9.0	1.55	4.0	5.2	5.7
pH 6.5-7.0	1.55	3.3	4.3	4.8
pH 4.0-4.5	1.40	3.8	5.3	5.9
January 12				
pH 8.5-9.0	1.30	5.1	6.5	7.4
pH 6.5-7.0	1.40	2.8	3.8	4.4
pH 4.0-4.5	1.25	8.8	8.6	9.7
February 1				
pH 8.5-9.0	1.50	4.5	6.8	7.6
pH 6.5-7.0	1.40	3.3	5.1	5.6
pH 4.0-4.5	1.45	4.5	6.9	7.8

The oxidase activity of the leaves was not influenced by the soil reaction or resulting growth. Catalase activity of the leaves showed the same relationship with reference to soil reaction and resulting growth as the green mature fruits, i. e., the least catalase activity occurred in the plants from plots with a soil reaction of pH 6.5-7.0. Measurements made on December 20 showed less catalase activity on all plots than those made on January 12 and February 1.

Since the soil reaction and resulting growth apparently had no effect on the oxidase activity of fruits or leaves for the fall crop no measurements were made on the spring crop. Catalase activity in the leaves was measured at two-week intervals, on March 30, April 12 and April 26. Determinations were made on the fruits on only one date, namely April 27. When leaf samples of March 30 and April 12 were taken only small green fruits were present in the first three clusters. Results are given in table VIII.

TABLE VIII. CATLASE ACTIVITY OF LEAVES AND FRUIT OF THE SPRING CROP OF TOMATOES.

Date	Sample	Soil Reaction	Catalase (c.c. of water displaced) Time in minutes		
			1	2	3
March 20	Leaves	pH 8.5-9.0	3.8	5.1	5.6
		pH 6.5-7.0	3.0	4.1	4.9
		pH 4.0-4.5	3.9	5.2	5.8
April 12	Leaves	pH 8.5-9.0	4.6	5.4	6.1
		pH 6.5-7.0	2.9	3.6	3.8
		pH 4.0-4.5	3.1	4.5	5.7
April 26	Leaves	pH 8.5-9.0	4.2	5.1	5.9
		pH 6.5-7.0	2.0	2.5	2.9
		pH 4.0-.4.5	4.1	5.2	5.8
April 27	Green Mature Fruit	pH 8.5-9.0	1.1	1.7	2.1
		pH 6.5-7.0	0.8	0.9	1.0
		pH 4.0-4.5	2.8	3.5	3.8
April 27	Ripe Fruit	pH 8.5-9.0	1.2	1.5	1.8
		pH 6.5-7.0	0.9	1.1	1.3
		pH 4.0-4.5	1.4	1.7	1.9
April 27	Green Fruit	pH 8.5-9.0	0.7	0.8	0.9
		pH 6.5-7.0	0.7	0.7	0.7
		pH 4.0-4.5	0.7	0.9	1.0

Results on catalase activity in the spring crop agree with those secured on the fall crop. Catalase activity of the leaves was less on the neutral plots (pH 6.5-7.0) and greater on the acid (pH 4.0-4.5) and alkaline (pH 8.5-9.0) plots. Both ripe, very green and green mature fruits showed the same relation to soil reaction and growth as the leaves, though the difference in the case of the very green fruit may be within the limits of experimental error. As with the fall crop, catalase activity was greater in the leaves than in the fruit.

DISCUSSION

The soils used in the plots were all of the same type, were uniformly prepared by composting and were thoroughly mixed in a highly efficient soil shredder; it was thought that this method would reduce the variable factors to a minimum. The use of soils with natural pH of the desired range was suggested. It was thought that the variations in fertility between the soils from various sources would increase the variable factors which influence growth.

The use of water or sand cultures with pH values of the desired range would no doubt have kept the variable factors to the minimum, but growth of the plants would not have been comparable to that secured when plants were grown in soil. Since this was a study of greenhouse soils, the conditions were more nearly comparable than with water or sand solutions.

By the addition of phosphoric acid to secure the acid reaction and hydrated lime to obtain the alkaline reaction, the calcium, hydrogen, hydroxyl and phosphate ions and the action of these on materials in the soil were factors which might affect the growth and yield of the plant. However, since the respective ions may cause acidity or alkalinity as measured by pH, the acidity or alkalinity of the soil was considered in general as the factor which caused variation in growth and yield.

Only the acid plot was treated with phosphoric acid, but available phosphorus was present in the soil in sufficient quantities on the neutral and alkaline plots to secure normal growth. Repeated tests and observations of plant growth in the compost used in the greenehouse benches showed that it contained enough of the essential elements for plant growth without the addition of any fertilizers. Hence, phosphorus was readily available on all the plots. The same was true for calcium added in the hydrated lime.

Sixteen plots in the greenhouse with soil reactions ranging from pH 3.5 to 9.0, used for another set of experiments, gave similar results with reference to yield and growth as the six plots included in this experiment. The largest yields and maximum growth were secured on the plots with a soil reaction which was neutral or slightly acid.

Van Alstine (18), Tarr and Noble (17), Duggar (6) and Appel (1) reported chlorotic effects from the use of nutrient solutions where the pH was on the alkaline side. This was said to be due to the insolubility of the iron. No such results occurred on the plants grown in the greenhouse plots in a soil with a similar reaction.

The data show clearly that there was a relation between the catalase activity and amount of growth and yield in the tomato. The differences in catalase activity of leaf tissue from plants growing in various soil reactions were greater than in the fruit. Catalase activity was much greater in the leaf tissue than in any stage of maturity of the fruit and may account for these greater differences.

The catalase activity in very green fruits was very low and consequently no consistent difference could be noted between fruits from the various plots. Catalase activity of the fruit appeared to be greatest in the green mature stage, and consistent differences due to conditions of growth were noted. As the fruit ripened catalase activity again decreased for fruits from all the plots.

Knott (13) found in spinach that when the plant changed from a vegetative to a reproductive type of growth the catalase activity decreased. In the experiments reported here yield and growth were associated on the neutral plot and catalase activity decreased under these conditions. Since production of fruit was high on the neutral plots, the type of growth secured could not be considered a vegetative type. The results reported here with respect to catalase activity were in accord with the results of Ezell and Crist (7), who found a negative correlation between yield and growth and catalase activity in the leaves of the apple. Heinicke (11) found catalase activity was reduced in the bark of the apple tree by fruiting. He concluded that growth-producing substances increased catalase activity, while substances which retarded vegetative activity had a retarding influence on such activity. Ezell and Crist (7) took exception to this, since their results, especially with lettuce, showed that, as growth increased, catalase activity decreased.

It appears from the present work that increased growth accompanied by increased yield may be associated with decreased catalase activity. Heinicke (11) considered only increased growth as associated with decreased catalase activity and disregarded yield of fruit as a factor.

No correlation was found in the present work between growth, yield and oxidase activity. Reed (15) found catalase and oxidase activity to be independent of each other, and this is substantiated here.

SUMMARY

Tomatoes grown on soil with a pH value of 6.5-7.0 gave higher yields and made greater growth as measured by total dry matter than tomatoes grown on soils of pH values of 4.0-4.5 and 8.5-9.0.

Catalase activity, growth and yield were negatively correlated in the vegetatively mature leaves, green mature fruit and ripe fruit. No apparent differences were observed in very green fruits.

In the tomato fruit catalase activity was lowest in very green fruits, much greater in the green mature stage, and became less in the ripe fruit.

Soil reaction and subsequent growth and yield had no apparent effect on oxidase activity, although oxidase activity was greater in the ripe fruits than in very green or green mature fruits.

Catalase and oxidase activity were apparently independent of each other.

LITERATURE CITED

1. Appel, M.
1918. Uber den Wert von der Croneschen Nährlosung. Zeitschr. Bot. 10:145-162.

2. Appleman, Chas. O.
1910. Some observations on catalase. Bot. Gaz. 50:182-192.

3. Auchter, E. C.
1923. Is there normally a cross-transfer of foods, water and mineral nutrients in woody plants? Md. Agr. Exp. Sta. Bul. 257.

4. Becking, L. G. M. B. and Hampton, H. C.
1920. Measurements of the catalyptic power of catalase. Amer. Jour. Bot. 7:261-274.

5. BIECHY, THEODOR.
1926. Können Fermentwirkungsmessungen zur Beurteilung der Vitalitat wichtiger Dulturpflanzen herangezogen werden? Fermentforschung 8:136-166.

6. DUGGAR, B. M.
1920. Hydrogen-ion concentration and the composition of nutrient solutions in relation to the growth of seed plants. Mo. Bot. Gardens, Ann. 7:1-49.

7. EZELL, B. D. AND CRIST, J. W.
1927. Effect of certain nutrient conditions on activity of catalase and oxidase. Mich. Agr. Exp. Sta. Tech. Bul. 78.

8. GRUSS, J.
1907. Enzymwirkungen am Wundrandt der Kartoffelknolle. Zeitschr. Pflanzenkrankheiten. 17:65-79.

9. HARVEY, R. B.
1920. An apparatus for measuring oxidase and catalase activity. Jour. Gen. Physiol. 2:253-254.

10. HEINICKE, A. J.
1924. Catalase activity in dormant apple twigs; its relation to the condition of the tissue, respiration and other factors. N. Y. (Cornell Univ.) Agr. Exp. Sta. Memoir 74.

11. .....
1923. Factors influencing catalase activity in apple leaf tissue. N. Y. (Cornell Univ.) Agr. Exp. Sta. Memoir 62.

12. .....
1926. The influence of fruiting on the catalase activity of the bark of apple trees. Amer. Soc. Hort. Sci. Proc. 23:263-269.

13. KNOTT, J. E.
1927. Catalase in relation to growth and to other changes in plant tissue. N. Y. (Cornell Univ.) Agr Exp. Sta. Memoir 106.

14. .....
1925. Some conditions influencing the determination of catalase activity in plant tissue. Amer. Soc. Hort. Sci. Proc. 398-407.

15. REED, G. B.
The relation between oxidase and catalase in plant tissue. Bot. Gaz. 62:409-412.

16. RHINE, L. E.
1924. Divergence of catalase and respiration in germination. Bot. Gaz. 78:46-67.

17. TARR, L. W. AND NOBLE, S. C.
1922. The effect of hydrogen-ion concentration upon the growth of the seedlings. Del. Agr. Exp. Sta. Bul. 131.

18. VAN ALSTINE, E.
1920. The interrelation between plant growth and the acidity of the nutrient solution. N. J. Agr. Exp. Sta. Rpt. 395-401.

NEW AND LITTLE KNOWN NEOTROPICAL TINGITIDAE

CARL J. DRAKE

From the Department of Zoology and Entomology, Iowa State College.

Accepted for publication September 25, 1928.

In the present paper two new genera, twenty-two species and one variety of Tingitidae are described from Mexico, West Indies, Central and South America. It is based upon material in the collection of the Carnegie Museum (Pittsburgh), Deutsches Entomologisches Institut der Kaiser-Wilhelm-Gesellschaft (Berlin-Dahlem), and the private collection of the writer. The writer is especially indebted to Doctors G. N. Wolcott, R. H. Van Zwaluwenburg, F. X. Williams, and Gregario Bondar for the gift of numerous specimens of Neotropical Tingitidae.

Nectocader, n. gen.

Head greatly prolonged, tumid, with four spines as in the genus *Cantacader;* bucculae very long, projecting considerably beyond apex of head, closed in front. Antennae long, slender; segments I and II swollen, short; III very long, slenderest; IV fusiform. Rostral channel very deep, long, open behind; rostrum extremely long, extending on the venter. Pronotum narrowed anteriorly, punctate, tumid, with five longitudinal carinae, the collum distinct. Paranota narrow, reticulate. Scutellum small, exposed, the posterior margin of pronotum not or scarcely produced. Elytra with a distinct clavus as well as costal, subcostal, discoidal and sutural areas; discoidal area very long, large, with prominent adventitious nervures; wings present.

Nectocader is most closely related to the genus *Cantacader* Amyot and Serville and belongs to the tribe *Cantacaderia* Stål. It may be distinguished from *Cantacader* by the more tumid head, subtruncate (either slightly excavated or slightly rounded) posterior margin of the pronotum and the exposed scutellum. Macropterous and brachypterous specimens are known to occur in one or perhaps two of the three described species.

Genotype, *Nectocader (Cantacader) gounellei* (Drake) from Brazil.

Nectocader germaini (Signoret)

Cantacader germaini Signoret, Ann. Soc. Ent., France, 1863, p. 586; Reed, Revista Chilena Hist. Nat. LV, 1902, p. — (reprint, p. 86).

Brachypterous female: Elongate-elliptical, body convex above, elytra (taken together) rounded behind. Margins of paranota and elytra serrate. Elytra a little longer than abdomen; costal area widest at base, uniseriate on distal half, with four to five rows of areolae at base and decreasing to one at middle, thence uniseriate; subcostal area very broad, with six to eight transverse costate nervures; discoidal area very large, long, with two prominent transverse nervures. Paranota narrow, composed of one

row of small round areolae, with two prominent teeth, one near the anterior end and the other at the middle. Rostrum reaching to the third venter. Antennae long, slender, the third segment distinctly shorter than in *N. tingitoides* (Spinola).

Chile, six macropterous females, collected by the late Dr. Carlos Reed. On account of the wider subcostal area and shorter antennae, it seems advisable to treat this species as distinct, rather than to consider it the macropterous female of *N. tingitoides*. As Signoret and Reed have both pointed out, *C. germaini* and *C. tingitoides* are not typical species of the genus *Cantacader* Amyot and Serville.

Nectocader tingitoides (Spinola)

Piesma tingitoides Spinola, in Gay, Hist. Chile, Zool., VII, 1852, p. 200.
Cantacader tingitoides Signoret, Ann. Soc. Ent., France, 1863, p. 575; Reed, Revista Chilena Hist. Nat. IV, 1902, p. — (reprint, p. 6).

A macropterous male from Chile is at hand. The antennae are about one-third longer than in the female specimens of *N. germaini*. The elytra are much longer than the abdomen. *Germaini* and *tingitoides* are very closely related, the former being based upon short winged females and the latter upon long winged males.

Nectocader gounellei (Drake)

Cantacader gounellei Drake, Bul. Brookl. Ent. Soc., XVIII, 1923, p. 81, fig. 1.

The extremely large size (6.4 mm. long and 3.6 mm. wide) and entirely uniseriate costal area distinguished this species at once from its congeners. The lateral margins of the paranota and elytra are not serrate. Known only from the type from Brazil.

Nyctotingis Drake, 1922

Nyctotingis Drake, Mem. Carn. Mus., IX, 1922, p. 362.
Orthotype, *Nyctotingis osborni* Drake.

Nyctotingis osborni Drake

Nyctotingis osborni Drake, Mem. Carn. Mus., IX, 1922, p. 363, fig. 1.

The male (*allotype*) is very similar to the female in size, form, color, and appearance. The abdomen is narrowed posteriorly, black, the clasper being dark brown and strongly curved. *Allotype* (male), Mera, Ecuador, Feb. 2, 1923, collected by Mr. F. X. Williams, in my collection. Heretofore known only from the *holotype* (female), Chapada, Brazil, in the Carnegie Museum.

Genus *Sphaerocysta* Stål, 1873

Sphaerocysta Stål, Enum. Hemip., III, 1873, pp. 120 and 128.
Logotype, *Sphaerocysta globiffera* (Stål).

Orifice long, prominent. Bucculae open or closed in front. Pronotum transversely swollen through the disc, punctate, uni- or tricarinate. Elytra

with a more or less prominent tumid elevation. Median carina either strongly raised and inflated at its apex or not raised at all. Discoidal area distinctly or indistinctly defined. Elytra a little longer than the abdomen. Nervures of hood, paranota and elytra moderately thick. Antennae and legs long, rather stout.

The pronotum is unicarinate in *S. fumosa*, n. sp. *S. egregia*, n. sp., differs from *S. fumosa* in having the paranota carina-like and non-reticulate behind and then expanded and reticulate in front; the median carina is neither raised nor inflated behind.

Sphaerocysta globiffera (Stål)

Tingis ? *globiffera* Stål, Rio Hemip., I, 1860, p. 65.
Sphaerocysta globiffera Stål, Enum. Hemip. III, 1873, p. 128.

Several specimens, Bahia, Brazil, collected by Gregario Bondar; one female, Sana Cruz, Brazil. According to the original descriptions and the subsequent notes published by Stål, the type of *S. globiffera* has a very narrow and uniseriate paranota. This character separates it at once from its nearest ally, *S. stali*, n. sp.

Sphaerocysta stali, n. sp.

Form, size, color and markings very similar to *S. globiffera* Stål, but differing in having a little broader and biseriate paranota, slightly smaller hood, and a little broader costal area. Pronotal and head character very similar to *S. globiffera;* tumid elevation between discoidal and subcostal areas slightly smaller; costal area irregularly uni- to biseriate, mostly uniseriate, with two rows at its widest part, the inner row very much smaller and mostly triangular in shape. Paranota narrow, strongly reflexed, biseriate, not widened behind. Rostrum reaching to the end of the mesosternum.

Length, 3.53 mm.; width, 1.35 mm.

Holotype, male, Rio Janeiro, in my collection. From the original description and notes in the Enumeratio Hemipterorum, it seems quite evident that Stål confounded this species with his *S. globiffera*. In fact, the type of *S. stali* was determined by Stål himself as *S. globiffera*. Champion (TRANS. ENT. SOC. LOND., 1898, p. 59, pl. II, fig. 11) illustrated *S. stali*, n. sp. instead of the true *S. globiffera* Stål.

Sphaerocysta inflata biseriata, n. var.

Differs from *S. inflata* Stål in having the costal area of the elytra much broader and entirely biseriate. The hood also seems to be slightly smaller and the tumid elevations on the elytra are a little larger. Examination of the type of *inflata* may prove this insect to be a distinct species. Pronotum, head, antennae and paranota as in *S. inflata*.

Length, 3.53 mm.; width, 1.35 mm.

Holotype, male, Chapada, Brazil, in Carnegie Museum.

Paratype, male, taken with type, in my collection.

Sphaerocysta egregia, n. sp.

Yellowish brown, the elytra slightly lighter and with a small brown spot on each tumid elevation. Pronotum considerably swollen thru disc, coarsely pitted, somewhat shiny. Hood subglobose, not so strongly raised as in *S. globiffera* Stål. Carinae parallel, distinct, but feebly raised, the median becoming almost obsolete behind. Paranota almost obsolete behind, carina-like; expanded in front, uniseriate, the marginal nervure very thick, the areolae small. Head brown, with two short, testaceous spines in front, each directed downward and slightly inward. Bucculae not very broad, open in front. Rostrum reaching slightly beyond the middle of the mesosternum. Antennae moderately long and rather stout; segment I much stouter and not quite twice as long as two; III three times as long as four.

Elytra rounded behind; costal area broad, mostly biseriate, triseriate at its widest part, the areolae large; discoidal area distinct, with five areolae at its widest part, the tumid elevations very small; subcostal area mostly biseriate; triseriate at its widest part, the areolae slightly smaller than those of discoidal area. Wings almost as long as the elytra.

Length, 2.6 mm.; width, 1.36 mm.

Holotype, male, Corumba, Brazil, in Carnegie Museum. The median carina and paranota distinguish *S. egregia,* n. sp. from its congeners.

Sphaerocysta fumosa, n. sp.

Pronotum transversely swollen thru the disc, narrowed anteriorly, unicarinate, the lateral carinae wanting; median carina formed as in *S. inflata* Stål, with one large cell in front of the strongly inflated posterior portion. Paranota biseriate, narrowed in front, rounded, the outer row of areolae very large. Hood subglobose, about the size and shape as in *S. globiffera* Stål, darker in color. Antennae with third and fourth segments wanting, the first segment a little over twice the length of the second. Elytra considerably longer than the abdomen, rounded behind; tumid elevations large, rounded, strongly inflated, costal area irregularly biseriate, areolae large and not of a uniform size; some of the transverse nervures fuscous; subcostal area biseriate. Wings a little shorter than the elytra. Rostrum reaching to the end of the mesosternum.

General color testaceous, with fuscous markings; pronotum fuscous. Nervures of hood and inflated portion of elytra dark; areolae of paranota and costal and sutural areas somewhat iridescent, hyaline. Body beneath brownish black. Legs yellowish brown.

Length, 3.21 mm.; width, 1.59 mm.

Holotype, female, Para, Brazil, in my collection. This species is most closely allied to *S. inflata* Stål, but readily separated from it by the larger and more strongly inflated tumid elevation of the elytra and the unicarinate pronotum.

Zatingis, n. gen.

Head short, with five spines. Bucculae broad, reticulate, closed in front. Antenniferous tubercles prominent, broad, strongly compressed laterally. Antennae long, slender; segment I longer and much stouter than two, the latter very short; III very long, moderately stout; IV fusiform. Pro-

notum tricarinate, the triangular process long. Hood moderately large, narrow, roof-like, projecting angularly between eyes. Paranota moderately reflexed, the antero-lateral corner terminating in a long slender spine. Orifice indistinct. Metasternum wide, the intermediate and posterior coxae widely separated, the intermediate pair being placed far back on the mesosternum and almost touching posterior coxae. Nervures large and prominent. Elytra considerably longer than the abdomen, obliquely rounded at apex; costal, subcostal, discoidal and sutural areas distinct and bounded by prominent costate nervures; discoidal area reaching beyond middle of elytra, deeply impressed, surrounded by a very prominent costate nervure. Wings present. Legs rather short and stout.

Genotype, *Zatingis extraria,* n. sp.

This genus is most closely related to the genus *Hormisdas* Distant of the Philippines, from which it may be separated by the much shorter and stouter legs, stouter antennae, broad antenniferous tubercles, prominent hood and much thicker nervures. The hood extends back of the collum to the transversely swollen portion of pronotum; the median carina unites with the median nervure of hood.

Zatingis extraria, n. sp.

Elongate, brownish testaceous, with fuscous markings, the nervures prominent. Head reddish brown; median and posterior spines very long, slender and sharp, longer than the anterior ones. Antennae very long, rather slender; segment I considerably swollen, constricted a little beyond middle, dark brown, distinctly thicker and almost two and a half times as long as the second; III extremely long, slightly curved, a little less than five times as long as four; yellowish brown; IV fusiform, thickest a little beyond the middle, blackish, except the basal portion yellowish brown. Rostrum extending between intermediate coxae.

Pronotum transversely swollen thru disc, very coarsely pitted, reticulate behind, tricarinate; carinae parallel, long, each composed of a single row of rather large areolae. Paranota moderately broad, irregularly biseriate, the antero-lateral margin terminating in a long, sharp spine. Hood moderately long, narrow, distinctly ∧-shaped, highest in front, strongly projecting ∧-like in front. Elytra moderately expanded, subangulate at apex; costal area moderately wide, biseriate, the areolae irregular in size and arrangement; subcostal area quadriseriate, the areolae fairly regular in size and rows; discoidal area reaching beyond the middle of elytra, distinctly raised along the outer margin, impressed along the inner margin, bounded by a very prominent costate nervure, the outer margin arcuate, somewhat narrowed and raised at apex. Wings longer than abdomen. Claspers strongly curved in male.

Length, 3.03 mm.; width, 1.36 mm.

Holotype, male, S. Bernardino, Paraguay, in my collection. The long terminal antero-lateral spines of the paranota separate this insect from any known American species.

Leptodictya Stål, 1873

Leptodictya Stål, Enum. Hemip., III, 1873, pp. 121, 127; Champion, Biol. Centr. Amer., Rhynch., II, 1897, p. 23.

Logotype, *Leptodictya ochropa* Stål.

Leptodictya approximata Stål.

Monanthia approximata Stål, Rio Hemip., I, 1860, p. 63.
Leptodictya approximata Stål Enum. Hemip., III, 1873, p. 127.

Female, Santa Paulo, Brazil, Jan. 28, 1923. This species has a much larger hood than *L. dohrnii* Stål or *L. ochropa* Stål. Antennae long, slender; segment I slightly thicker and less than twice as long as two, dark fuscous, concolorous with two and four; III long, slender, pale brown, two and a half times as long as four. Legs pale brown. Elytra widening posteriorly, prominently marked with dark fuscous, the tips separated, the lateral margins finely serrate.

Leptodictya vulgata, n. sp.

Form and size similar to *L. approximata* Stål. Antennae long, slender, black, clothed with bristly hairs; segment I a little stouter and practically twice as long as two; III very long, two and three-fourths times as long as four. Head with five long, porrect, sharp, testaceous spines, the median not quite reaching the tip of first antennal segment. Rostrum reaching to intermediate coxae. Legs long, slender, brownish fuscous, the tips of femora and tarsi blackish. Pronotum considerably swollen thru disc, dark brown to black, becoming testaceous on triangular portion, frequently covered (also head) with whitish exudations, tricarinate, each carina composed of a single row of small areolae; median carina a little more elevated and distinctly raised in front. Hood pale testaceous, rather large, considerably longer than wide, projecting anteriorly (between eyes) almost to apex of head. Paranota pale testaceous, sub-inflated, much wider beneath and more strongly reflexed than in *L. approximata*.

Elytra widening posteriorly, the tips separated, the outer margins finely serrate, the areolae hyaline; costal area broad, the areolae irregular in size and arrangement; testaceous, the outer half of four obliquely transverse nervures, the nervures forming marginal row of areolae and all nervures on distal half of elytra brown to fuscous, the marginal nervure dark fuscous; subcostal area pale testaceous, biseriate; discoidal area pale testaceous, long, narrow at base and apex, with four confused rows of areolae at widest part; nervures of sutural area fuscous. Male genital segments broad, the claspers strongly curved. Body beneath black.

Length, 4.71 mm.; width, 2.1 mm.

Holotype, male, Naranjapata, Ecuador, elevation 1800 ft., Dec., 1922, collected by F. X. Williams, in my collection; 3 paratypes (males), Huigra, Ecuador, elevation 4000 ft., collected by F. X. Williams. The color of antennae and semi-inflated and very strongly reflexed paranota distinguish this insect from *L. approximata* Stål.

Leptodictya championi, n. sp.

Moderately elongate, the color and markings similar to *L. cretata* Champion. Head black, spines testaceous; anterior pair longest, sharp, porrect, considerably shorter than the first antennal segment. Antennae long, slender, brownish black; segment I three times as long as two, the latter short; III very long, a little more than four times as long as four.

Pronotum slightly more tumid than in *L. cretata;* hood distinctly larger, more inflated and rounded, the median nervure fuscous; paranota more strongly reflexed, the turned over portion narrower, widest at middle, biseriate. Discoidal area much narrower, with four or five areolae at its widest part; other characters of elytra similar to *L. cretata.* Body beneath black.

Length, 4.41 mm.; width, 2.35 mm.

Holotype (male) Purula, Vera Paz, Guatemala, collected by G. C. Champion, in my collection. In the BIOL. CENTR.-AMER., II, 1897, p. 24, Champion confused this species with *L. cretata* Champ. from Panajachel. The shorter spines on the head, the dark antennae, larger and differently shaped hood and narrower discoidal area separate *L. championi,* n. sp., from *L. cretata* Champion.

Two cotypes of Champion's *L. cretata* from Panajachel are at hand; the paranota are not so strongly reflexed and wider than in *L. championi,* triseriate above, widest in front of middle. These paratypes agree with the original description and figure of *L. cretata.*

Leptodictya madelinae, n. sp.

Similar to *L. championi,* n. sp. in size, color and form. Head with five moderately long, stout, blunt, testaceous spines; black, covered with white exudations. Antennae long, slender, brownish black; segment I stouter and twice as long as two, the latter considerably embrowned; III long, twice as long as four, the latter much longer than one and two conjoined. Pronotum black, coarsely pitted, more strongly swollen, the hood slightly smaller, and somewhat similar in form to *L. championi;* paranota slightly broader, mostly biseriate, sometimes with a few additional cells. Elytra a little darker than in *L. championi;* costal area unevenly reticulate; subcostal area irregularly biseriate; discoidal area long, narrow, impressed, with four somewhat confused rows of areolae at its widest part. Wings smoky, longer than the abdomen. Legs dark brown; tarsi black. Rostrum reaching to the intermediate coxae.

Length, 4.34 mm.; width, 2.34 mm.

Holotype, male, Banos, Ecuador, S. A., Jan. 8, 1923, collected by F. X. Williams, in my collection. *Paratypes,* 2 males, taken with type. The rostrum is much shorter and the fourth antennal segment (52) is much longer in this species than in *L. championi* (34).

Leptodictya williamsi, n. sp.

Narrower than *L. grandatis,* n. sp., the elytra marked with brown instead of dark fuscous. Head covered with whitish exudations, with five moderately slender spines, the anterior pair about one-third as long as the basal segment of antennae. Antennae long, slender, fuscous-black; segment I a little stouter and twice as long as two; III nearly two and a half times as long as four. Hood much narrower than in *L. grandatis,* testaceous. Pronotum black, with whitish exudations, punctate, considerably swollen; paranota similar to *L. grandatis,* the margin touching the pronotum a little straighter. Median carina not as strongly raised anteriorly as in *L. grandatis.* Rostrum extending to the metasternum. Legs long, brown.

Elytra more widely reticulated than in *L. grandatis,* the costal and discoidal areas narrower; subcostal area biseriate, vertical; costal area irregularly and unevenly reticulated, with six to seven cells at its widest part. Wings smoky, longer than abdomen.

Length, 4.70 mm.; width, 2.00 mm.

Holotype, male, and *allotype,* female, Banos, 6000 feet elevation, Ecuador, Oct. 26, 1922, collected by Dr. F. X. Williams, in my collection. Five paratypes, taken with type. The nervures of sutural, subcostal and distal half of costal areas (also one or two rows along outer margin) are brown. The areolae are hyaline.

Leptodictya colombiana, n. sp.

Resembles *L. williamsi* and *L. grandatis,* but separated at once from them by the much smaller hood. Pronotum strongly swollen thru disc, coarsely punctate, tricarinate, each carina composed of one row of areolae. Hood very narrow, small, testaceous, projecting angulately anteriorly. Paranota biseriate above, mostly triseriate below, similar in shape to *L. williamsi.* Antennae blackish fuscous, segment I scarcely twice as long as two; III two and a half times as long as four. The median carina slightly more raised and considerably raised anteriorly. Head black, with five short spines. Elytra broad, testaceous, the nervures along the outer border and in distal half brown, the areolae hyaline, the outer marginal nervure larger, fuscous, and indistinctly serrate; subcostal area vertical, biseriate; discoidal area long, narrow, narrowed at base and apex, with four rows of areolae at its widest part. Legs brown, the tips of femora and tarsi black.

Length, 5.46 mm.; width, 2.99 mm.

Holotype (male), Colombia, S. A., March 11, 1912, H. S. Parish, in my collection. The very small and narrowly /\-shaped hood distinguishes this species from its allies. Its hood is smaller and much narrower than in *L. dohrnii* Stål.

Leptodictya sodalatis, n. sp.

Large, yellowish brown, some of the veinlets and transverse veins dark brown or fuscous, the areolae hyaline. Form similar to *L. leinahoni* Kirk., but much smaller, lighter in color, the hood very much smaller. Head brown, with five very long, sharp, testaceous spines, the anterior pair reaching a little beyond the second antennal segment. Rostral channel deep, slightly widening posteriorly, the rostrum reaching almost to the end of the channel. Antennae long, slender, pale brown; segment I rather long, a little less than four times as long as two; II very short; III very long, more than five times as long as one; IV long, subequal to one in length, the distal five-sixths fuscous or black.

Pronotum strongly narrowed anteriorly, brown, moderately swollen thru the disc; strongly narrowed anteriorly, punctate; carinae parallel, each composed of a single row of minute areolae, the median carina more strongly rasied and biseriate in front. Paranota very broad in front, much narrower behind; outer margin nearly straight, armed with a few spines; reflexed portion composed of a single row of large rectangular areolae; in front composed of five rows of cells. Hood narrow, small, /\-shaped as

viewed from above, projecting faintly over the base of the head. Collum distinctly areolate, the calli smooth and shiny. Body beneath brown. Elytra broad, much longer than the abdomen, broadly rounded at the apex; the lateral margins slightly rounded, bluntly serrate, each small tooth-like protuberance terminating in a stiff bristle-like hair; costal area brown, with many confused rows of large areolae (seven or eight rows at widest part); with three or four cross nervures somewhat costate and fuscous: discoidal area long, extending beyond the middle of the elytra, with six rows of areolae at its widest part, the sides slightly rounded, the adventitious nervure short, running a little beyond the middle obliquely across discoidal area; nervure separating areas with erect, moderately long hairs, the other nervures with a few scattered haris. Wings greatly reduced.

Length, 4.9 mm.; width, 2.8 mm.

Holotype (male) and *allotype* (female), Cochahamba, Bolivia, in my collection. *Paratype,* four specimens, collected with type. Two paratypes in collection of Deutsches Entomologisches Institut der Kaiser-Wilhelm-Gessellschaft.

Leptodictya luculenta, n. sp.

Elongate, broad, pale cinnamon brown, the paranota and costal area a little lighter, areolae hyaline. Head brown, tumid, with very long sharp spines, the posterior pair very long. Rostral channel widening posteriorly, open behind; rostrum reaching a little beyond the mesosternum. Paranota moderately broad, the reflexed lateral margin slightly emarginate; overlapping portion broadest in front of middle, biseriate, the outer margin rounded and resting on pronotum. Pronotum considerably swollen thru disc, deeply and coarsely pitted, becoming reticulate in triangular portion, tricarinate, each carina composed of a single row of small areolae; median carina more strongly raised, biseriate in front; lateral carinae parallel, slightly less raised. Hood Λ-shaped, projecting faintly in front, larger than in *L. dohrnii* Stål.

Elytra broad, rounded behind, the tips separated; costal area very broad, with two or three large transverse nervures, with five to six rows of areolae at its widest part, the areolae moderately large and a little variable in size and arrangement; subcostal area very narrow, uniseriate; discoidal area long, narrowed at base and apex and composed of six rows of small somewhat rounded areolae at its widest part. Body beneath dark brown, becoming darker on genital segments. Legs long, rather slender, brown, the tips of tarsi dark. Antennae long, slender, brown; segment I a little stouter, two and one-half times as long as two; III very long, slightly more than twice as long as four; IV becoming black on the distal two-thirds, clothed with numerous pale hairs.

Length, 4.44 mm.; width, 1.78 mm.

Holotype, male, and *allotype,* female, Mera, Ecuador, collected by F. X. Williams, in my collection. This species resembles *L. evidens,* n. sp., from which it may be separated by the characters given below.

Leptodictya evidens, n. sp.

A little broader and slightly lighter in color than *L. luculenta,* the paranota, inner portion of costal and sutural areas and discoidal area

mostly brownish testaceous. Head reddish brown, the spines moderately long, sharp, brownish testaceous; anterior spines reaching a little beyond the middle of first antennal segment. Antennae long, brown; segment I twice as long and a little stouter than two; III three times as long as four, the latter blackish and clothed with pale brown, carinae parallel, other characters as in *L. luculenta.* Hood considerably larger, broader, and a little more produced than in *L. luculenta.* Rostrum extending almost to apex of sulcus. Paranota formed somewhat as in *L. approximata* Stål, mostly biseriate, a few extra cells at its widest part, the outer reflexed margin rounded. Elytra very similar to *L. luculenta,* but a little broader, more broadly rounded behind and lighter in color; wing reaching to tip of abdomen.

Length, 4.41 mm.; width, 1.89 mm.

Holotype, female, Tabernilla, Panama, in my collection. *Paratype,* females, taken with type. The much larger hood and rounded outer reflexed margins of the paranota distinguish this species from *L. luculenta,* n. sp.

Leptodictya formosatis, n. sp.

Head black; median and anterior spines rather short, blunt, testaceous, porrect; posterior pair sharp, a little longer, contiguous with the head. Antennae long, brownish black; segment I moderately long, constricted a little beyond the middle, stouter and not quite twice as long as two; III long, not quite three times as long as four; IV longer than one and two taken together, the hairs pale, much thicker and longer. Rostrum reaching almost to the apex of sulcus. Pronotum very strongly swollen thru disc, coarsely punctate, black, shiny, tricarinate, each carina composed of a single row of very small areolae. Paranota narrow, reflexed margin rounded and fuscous, the distal portions of anterior and posterior femora, anterior tibiae and tarsi black.

Elytra prominently marked with fuscous, the marginal nervure fuscous, broadly rounded at apex; costal area broad, the areolae very irregular in size and arrangement, with six or seven areolae at its widest part, brown, a few areolae at base and two to three rows along subcostal area; the rest of the surface brown except four depressed oblique nervures and areolae in these depressions, the other areolae hyaline; subcostal area biseriate, brown at base and becoming testaceous toward apex; discoidal area long, broad, dark fuscous, an indistinct spot beyond middle and a spot at apex testaceous; nervures of sutural area dark fuscous; the areolae subhyaline. Wings scarcely longer than abdomen.

Length, 5.51 mm.; width, 2.6 mm.

Holotype, female, and *allotype,* male, Mera, Ecuador, Feb. 2, 1923, taken by F. X. Williams, in my collection. *Paratype,* female taken with type. Allied to *L. approximata* Stål, but distinguished by smaller hood, more swollen pronotum, broader discoidal area and more prominent color markings.

Leptodictya fusca, n. sp.

Moderately large, dark fuscous, the paranota and hood lighter. Antennae long, slender, dark testaceous; segment I dark fuscous, a little

thicker and about one and a half times as long as two; III very slender, straight, long, a little less than three times as long as four. Rostrum extending to the metasternum. Bucculae closed in front. Head short, black, with five long, slender, sharp spines.

Pronotum strongly swollen thru disc, coarsely pitted, reticulate at apex, tricarinate; carinae parallel, thin, each composed of a single row of very small cells. Hood moderately large, projecting subangularly in front. Paranota with two to three rows of areolae on the reflexed portion. Elytra broadly expanded, rather indistinctly serrate along the outer margins, entirely dark fuscous in color; costal area very broad, with four enlarged oblique nervures, about nine or ten areolae at its widest part, the areolae not definitely arranged in rows; subcostal area narrow, biseriate; discoidal area very long, narrowed at base and apex, reaching beyond the middle of elytra, without adventitious nervures, with six to seven rows of areolae at its widest part. Wings a little shorter than elytra. Body beneath dark yellowish brown. Legs pale testaceous, the tarsi darker.

Length, 3 mm.; width, 1.8 mm.

Holotype, male, Panama Canal Zone, in my collection. The dark fuscous color (areolae clouded with fuscous) separates this species from allied forms. A small area at the widest part of costal area has a tendency to become a little paler.

Australotingis Hacker, 1927

Australotingis Hacker, Mem. Queensland Mus., IX, 1927, p. 29.
Orthotype, *Australotingis franzeni* Hacker.

Australotingis williamsi, n. sp.

Very broad, blackish fuscous, the expanded portion of elytra yellowish brown and with prominent, broad, oblique, fuscous bands. Head, pronotum, hood, paranota, and discoidal and subcostal areas of elytra with whitish exudations. Bucculae prominent, reticulate, closed in front. Head short, black, the three frontal spines long, sharp, dark brown; posterior spines much shorter and slenderer. Antennae long, slender, clothed with a few long hairs; segment I rather stout, a little shorter than the distance between eyes, slightly curved, slightly constricted before apex, brownish black; II practically one-half the length of one, brownish black; III long, slender, a little more than twice as long as four, almost straight, brownish, slightly darker at base and the apical one-fourth blackish; IV long, slightly stouter than III, clothed with numerous hairs of various lengths, twice as long as one. Antenniferous tubercles greatly flattened laterally, wide, not prominent. Rostral channel open behind; rostrum broken, apparently reaching a little beyond the intermediate coxae. Orifice prominent. Legs dark reddish brown, the tarsi black. Body beneath black, the last segment of venter and genital segments somewhat embrowned. Hood, pronotum, paranota and elytra clothed with numerous long, very fine, erect or nearly erect, whitish to yellowish white hairs.

Pronotum transversely swollen thru disc, coarsely punctate; tricarinate; lateral carinae parallel, long, prominent, about one-third as high as the median, composed of one row of areolae; median carina strongly foliaceous, composed of a single row of long rectangular areolae. Hood mod-

crately large, strongly inflated, extending over base of head, not twice as high as the median carina. Paranota long, strongly inflated, hood-like in appearance, the hairs more numerous along the sides, the outer margin resting near the lateral carina. Elytra broad, broadly rounded behind, the tips separated. Costal area broad, with three depressed, slightly oblique, fuscous bands (both nervures and areolae fuscous), with six to seven rows of areolae at its widest part, very irregularly reticulate; subcostal areas biseriate; discoidal area long, extending almost to apex of abdomen, narrowed at both base and apex.

Length, 5.14 mm.; width, 2.41 mm.

Holotype, female, Mera, Ecuador, Feb. 2, 1923, F. X. Williams, collector, in my collection. Named in honor of Dr. Williams, who has collected a large number of interesting Tingitidae in South America. This is the first record of this genus in America, recorded heretofore only from Australia.

Genus *Leptopharsa* Stål, 1873

Leptostyla Stål, Enum. Hemip., III, 1873, pp. 120 and 125.
Leptopharsa Stål, Enum. Hemip., III, 1873, pp. 122 and 126.
Gelchossa Kirkaldy, The Entomologist, XXXVII, 1904, p. 280.
Leptostyla Champion, Biol. Centr.-Amer., Rhynch., II, 1897, p. 11.
Leptopharsa Champion, Biol. Centr.-Amer., Rhynch., II, 1897, p. 21.
Gelchossa Drake, Mem. Carn. Mus., IX, 1922, p. 372.
Leptopharsa Drake, Mem. Carn. Mus., IX, 1922, p. 370.
Leptopharsa Drake, Proc. Biol.. Soc. Washington, Vol. 41, 1928, pp. 21-24.

Leptopharsa tenuatis n. sp.

Elongate, very slender, testaceous, the disc of pronotum and some of the veinlets of sutural area brown. Head black, short; posterior spines long, slender, curved, testaceous; median spine stouter, straight, porrect, sharp; anterior pair wanting. Rostral sulcus closed behind, the rostrum extending to the middle of mesosternum. Pronotum slightly swollen thru disc, moderately narrowed anteriorly, distinctly pitted; triangular process long, testaceous, reticulate; tricarinate, each carina uniseriate, testaceous, the lateral ones slightly converging posteriorly. Paranota strongly reflexed, testaceous, narrow, biseriate, the basal row of cells very small. Hood very small, very narrow, faintly projecting anteriorly.

Elytra long, very narrow, the lateral margins nearly straight; costal area reflexed, moderately wide, biseriate; subcostal area much narrower, biseriate; discoidal area elongate, with three to four rows of areolae at its widest part. Wings smoky, much longer than the abdomen; legs long, slender, brownish testaceous. Antennae very long, slender; segment I very long, fuscous, almost four times as long as two, broadly constricted near apex; II short, fuscous; III very long, pale brown. Body beneath black.

Length 3.00 mm.; width, .71 mm.

Holotype, male, Brazil, in my collectoin. This species is most closely allied to *L. longula* Drake, *L. manihotae* Drake and *L. illudens* Drake, but differs in the characters of antennae, spines on head, and pronotal carinae. It is also shorter than *L. longula.*

Leptopharsa distantis n. sp.

Moderately elongate, testaceous; the head and pronotum brownish. Head with five moderately stout, blunt, pale brown spines, the posterior pair curved inwardly and contiguous with the head; the median and anterior pair directed obliquely downward. Segment I of antennae thicker and slightly more than twice as long as two; III and IV wanting. Rostral channel widening posteriorly, very wide on the metasternum; rostrum extending to the intermediate coxae. Hood very small, faintly projecting in front, somewhat transverse.

Body beneath black. Legs moderately long, yellowish brown, the tarsi darker. Pronotum moderately swollen thru disc, coarsely pitted, the apex of triangular process yellowish; lateral carinae faintly converging in front and behind, indistinctly areolate; median carina slightly more elevated, composed of a single row of tiny areolae. Paranota moderately expanded, slightly reflexed, biseriate, the outer margin broadly rounded. Elytra considerably longer than abdomen; rounded behind; costal composed of two regular rows of large areolae; subcostal area about as wide, but composed of four rows of small areolae; discoidal raised, extending beyond middle of elytra, narrowed at both base and apex, with five or six rows of areolae at its widest part, the areolae a little larger than those of subcostal area. Wings a little shorter than the elytra.

Length, 2.51 mm.; width, 1.14 mm.

Holotype, male, Tamasopa, Mexico, Dec. 4, 1909, in my collection. This species belongs to the division of the genus *Leptopharsa* Stål, having a transverse hood. It is shorter and broader than *L. elegantula* Stål.

Leptopharsa celebratis, n. sp.

Elongate, narrow, becoming a little wider posteriorly. Head short, black; posterior spines very short, blunt, testaceous, resting on the head; other spines wanting. Antennae long, slender, fuscous-black; segment I slightly constricted a little beyond the middle, moderately long, considerably stouter and a little more than twice as long as two; III very long, slender; IV wanting. Bucculae contiguous at the base in front. Rostrum rather short, reaching to a little beyond the prosternum.

Pronotum considerably swollen thru disc, coarsely and deeply pitted, narrowed anteriorly, tricarinate, black; median carina raised in front forming a small transverse hood; lateral carinae practically parallel, faintly curved inwardly in front. The apex of triangular portion and carinae somewhat tinged with testaceous. Paranota narrow, testaceous, slightly reflexed, the outer margin straight, a little wider and biseriate in front, uniseriate behind, the areolae rather small. Elytra rounded behind, nervures separating areas costate, prominent, and tinged with dark reddish brown; costal area testaceous, moderately broad, composed of two complete rows of areolae and a partial third series at its widest part, the areolae moderately large and sub-hyaline; subcostal area biseriate, nearly vertical, black; discoidal area long, narrowed at base and apex, widest at the middle, and there with five rows of areolae, black; sutural area with the areolae becoming larger distally, brownish black. The areolae of subcostal, discoidal and sutural areas whitish or smoky, non-transparent. Legs long, rather slender, reddish brown, the tip of tarsi darker.

Length, 3.51 mm.; width, 1.14 mm.

Holotype, male, and *allotype*, female, Rio Grande du Sul, Brazil, in my collection.

Leptopharsa calopa, n. sp.

Moderately elongate, narrow. Head short, black, with five long, slender porrect spines; median and anterior spines very long and brown, the posterior pair shorter and whitish. Rostral channel widening posteriorly, open behind, the rostrum not quite extending to the middle of the mesosternum. Legs slender, testaceous, the tarsi brown. Antennae very long, slender, testaceous; segment I short, slightly stouter and twice as long as two, tinged with brown; III very long, two times as long as four; IV long, twice as long as one and two conjoined.

Pronotum considerably swollen thru disc, coarsely pitted, smooth, brownish black, the posterior process pale testaceous and reticulate. Hood very small, pale testaceous, projecting sub-triangularly in front. Carinae foliaceous, pale testaceous, uniseriate; median carina slightly more raised; lateral carinae, long, faintly constricted behind the disc. Paranota moderately wide, entirely biseriate, the basal row of areolae very small. Elytra considerably longer than abdomen, rounded behind, the tips separated, the nervures fuscous, except the basal two-thirds of costal area pale testaceous; costal area biseriate at base, thence triseriate; subcostal area narrow, subvertical, biseriate; discoidal area narrow, narrowed at both base and apex, impressed, not quite reaching the middle of elytra; wings about as long as the abdomen. Body beneath black.

Length, 2.59 mm.; width, 1.12 mm.

Holotype, male, Chapada, Brazil, collecetd by H. H. Smith, in Carnegie Museum. This species resembles *L. divisa* (Champion), but may be separated from it by the longer antennae and costal area of elytra.

Leptopharsa distinconis, n. sp.

Head with a stout, blunt, strongly curved downward, median spine. Bucculae closed in front, the rostrum reaching to the end of the rostral sulcus. Antennae very long, slender, indistinctly pilose; segment I very long, five times as long as two, very widely constricted in front of apex, brownish black; II and III testaceous, the latter not quite one and a half times as long as four; IV very long, slender, brownish black, longer than one and two conjoined. Legs long, slender.

Pronotum black, becoming lighter toward apex of triangular process, moderately swollen on disc; median carinae very strongly raised, long, nearly twice as long as hood, broadly arched above, at its highest point more elevated than hood, composed of one row of very long rectangular areolae. Hood moderately large, not extending to the middle of the disc, projecting anteriorly to the apex of head, the eyes not concealed. Lateral carinae strongly foliaceous, long, constructed behind middle, each composed of a single row of large areolae. Elytra long, divergent, widening posteriorly, constricted beyond the middle, rounded at the tip; areolae hyaline, the costal area broad, with four or five somewhat irregular rows of areolae at its widest part; subcostal area vertical, mostly triseriate; discoidal area short, not reaching middle of elytra, narrowed at both base and apex. Nervures of discoidal, subcostal, three or four oblique nervures of costal area, and a long oblique curved fascia (including cells) extending

from just behind discoidal area to tip of each elytron, dark brown. Wings slightly longer than abdomen.

Length, 3.52 mm.; width, 1.40 mm.

Holotype, male, and *allotype,* female, Chapada, Brazil, collected by H. H. Smith, in Carnegie Museum. *Paratypes,* 11 specimens, collected with type, in Carnegie Museum and my collection. This species is most closely allied to *L. vesiculosa* (Champ.), from which it differs in the characters of the hood, paranota, and carinae.

Leptopharsa peruensis, n. sp.

Form and general appearance similar to *L. distinconsis,* n. sp.; color much darker, the nervures brown to fuscous. Antennae very long, slender; segment I very long, attenuated toward apex, five times as long as two, black; III testaceous, nearly one and three-fourths times as long as four, the latter very long and black. Head black, the median spine projecting almost directly upward, moderately long. Hood large, dark, slightly longer than high, not projecting as far forward as in *L. distinconsis.* Pronotum black, the triangular portion short and narrow; median carinae very strongly foliaceous, not quite as high as hood, composed of one row of very long, rectangular areolae; lateral carinae considerably raised, uniseriate, slightly constricted behind the middle.

Elytra widening posterioly, divaricating behind, with a prominent depressed, oblique, fuscous band at the apex of costal area; costal area broad, with five irregular rows of areolae at its widest part; subcostal area almost vertical, triseriate; discoidal area short, strongly raised toward the outer margin, almost triangular, the posterior margin almost oblique. Wings scarcely as long as abdomen. Body beneath black. Legs long, slender, yellowish brown.

Length, 3.57 mm.; width, 1.46 mm.

Holotype, female, Peru, in my collection. The dark veins, hood, and discoidal area distinguish this insect at once from the species having a very long basal segment of antennae. The paranota are large, strongly reflexed, widest opposite humeri and each is composed of three rows of large areolae.

Leptopharsa walcotti, n. sp.

Allied to *L. tumida* (Champion), but larger and with the median carina strongly and sharply arched behind the hood. Antennae long, testaceous; segment I long, slightly stouter and three times as long as two; III a little more than three and a half times as long as four. Hood very large, strongly inflated, not concealing carinae. Paranota very wide, strongly reflexed, with five rows of areolae at its widest part, the anterior and posterior margins slightly recurved; lateral carinae extremely short, each somewhat resembling a long spine, not concealed by the hood. Elytra widening posteriorly, the tips separated, similar in shape to *L. tumida,* the long oblique curved fascia faintly colored, the areolae hyaline; costal area widely reticulated, with four rows of areolae at its widest part. Rostral channel open behind, the rostrum extending to its apex. Lateral margins of elytra finely serrate.

Length, 3.7 mm.; width, 1.73 mm.

Holotype (male), and *allotype* (female) Gonaivets, Haiti, Feb. 19, 1900, collected by G. N. Wolcott, in my collection. *Paratypes*, 9 specimens, taken with type. This insect is named in honor of Dr. Wolcott, who has taken a keen interest in the insect fauna of Haiti.

Leptopharsa cubana, n. sp.

Very similar in form, color and appearance to *L. tumida* (Champion), but much smaller and with the median carina exposed and distinctly arched behind the hood. Hood moderately large, about one-half as large as in *L. wolcotti;* paranota similar in form, but much smaller, with three or four rows of areolae at its widest part, the outer margin finely serrate; subcostal area vertical, mostly biseriate. Head with the median spine porrect, straight, much longer and stouter than the others. First segment of antennae brownish, stouter and three times as long as two; III and IV wanting. Rostrum reaching to the end of the rostral groove.

Length, 2.44 mm.; width, 1.19 mm.

Holotype, male, and *allotype*, female, Peninsula de Guanahacabibes, Cuba, 1924, in my collection. The color and markings of this species are quite similar to *L. tumida;* the different median carina and the smaller hood separate it at once from either *L. wolcotti* or *L. tumida*.

THE FERMENTATION OF CORNSTALKS AND THEIR CONSTITUENTS*

*I. Studies on the Pectin-Fermenting Bacteria***.

LLOYD ALLEN BURKEY**

From the Department of Bacteriology, Iowa State College.

Accepted for publication September 20, 1928.

Organisms capable of fermenting pectin or related compounds in plant tissues have been studied principally by investigators interested in the retting of flax, hemp, and other plants. These studies have been made in a large measure from the standpoint of commercial retting. In only a few cases has any attention been paid to the systematic relationships of the organisms producing the change.

A Committee on Nomenclature of Pectin (1927) has recommended the use of the name "pectin" for the soluble compound and "protopectin" for the insoluble compound occurring in plant tissue. In referring to these substances in plant tissue the term "natural pectin" will be used.

The use of plant tissue as a substrate for the isolation and identification of pectin-fermenting bacteria has proved to be an unreliable procedure due to variation in content and in composition of the natural pectin in different plants. Furthermore, the disintegration of plant tissue when acted upon by these bacteria may be influenced by other factors, such as the maturity of the plant and the absence of suitable conditions for the growth of the organisms.

The use of a chemically pure pectin for the isolation and identification of pectin-fermenting bacteria should prove advantageous over the use of plant tissue. At present pectin may be obtained which is approximately pure and is reasonably constant in composition. It may not be identical with the pectin as it occurs in plant tissue, but its use makes possible an improvement in technique.

The early investigations of pectin date from the discovery and naming of pectin by Braconnot (1825), who separated it from fruit juices. Payen (1856) and Mangin (1889) studied the pectin which occurs naturally in plant tissue. They concluded that pectin in some form constitutes a large part of the middle lamella. Later Mangin (1893) showed the effect of acids and alkalies on the natural pectin in plant tissue. He also studied the solvent action of ammonium citrate, oxalate, tartrate, and the salts of other organic acids. These he observed to form double salts with the pectin. He concluded that the middle lamella of plant tissue consisted largely of a

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*Submitted to the Graduate Faculty of the Iowa State College in partial fulfillment of the requirements for the degree Doctor of Philosophy in Bacteriology.

**The author wishes to acknowledge his indebtedness to Dr. C. H. Werkman for valuable advice and assistance given throughout the course of this work, to Dr. E. I. Fulmer and Dr. R. M. Hixon for valuable assistance in relation to the chemistry of pectin.

calcium salt of pectic acid. Furthermore, he showed the middle lamella to be stained by ruthenium red, in this respect resembling many plant slimes and gums. Beijerinck (1904) referred to the natural pectin as pectose. Tschirch (1908) separated a material which he considered to be the mother substance of pectin and named it protopectin.

Studies on the fermentation of pectin date from the discovery of the enzymes pectase and pectinase. The former was obtained by Frémy (1848). It caused the gelation of the pectin solution. The gel formation was considered to be due to the hydrolysis of pectin to pectic acid. Later, Bertrand and Mallérve (1894) showed that pectase is widely distributed among vegetables. Their investigations further indicate that gel formation is influenced by acidity and the presence of a salt of calcium, barium or strontium. Von Fellenberg (1918) has recently shown that sucrose is necessary for the gel formation in fruit juice.

Another important enzyme connected with the fermentation of pectin is pectinase. This was identified in malt extract by Bourquelot and Hérissey (1898). It hydrolyzes pectin with the formation of simple sugars and acids. The work of Beijerinck and Van Delden (1903) showed it to produce marked retting of plant tissue. They termed it pectosinase. It is probably identical with the pectinase isolated by Bourquelot and Hérissey (1898). Later studies on the pectin-fermenting enzyme by Jones (1905) and others have shown that the organisms causing the soft rots of vegetables produce pectinase capable of attacking the natural pectin of the middle lamella. However, if the name "protopectin" is to be used for the insoluble pectin occurring in plant tissue, the enzyme attacking such substance may well be called protopectinase.

The relation of pectin fermentation to the retting of flax and hemp was recognized by Kolb in 1868. He showed that the fibers of plants were held together by the natural pectin corresponding to the pectose studied by Frémy (1848), and that the fermentation occurring during the retting process resulted in the formation of pectic acid. He believed retting to be the result of the fermentation of the natural pectin. However, other investigators interpreted their results as showing that retting was the result of cellulose decomposition. In 1877 Van Tieghem isolated an organism which he named *Bacillus amylobacter* and which he believed to be responsible for the retting of plant stems because of its ability to decompose cellulose. Mangin (1891) concluded the natural pectin to be associated with cellulose in the plant and considered the fermentation of pectin to be correlated with that of cellulose. Winogradsky and Fribes (1895) showed that the loss in weight of flax stems and sugar beet slices following the retting fermentation corresponded very closely to the original pectin content. Furthermore, they showed that the cellulose of plant stems was unchanged as the result of the usual retting process. Their results confirmed the findings of Kolb and established pectin fermentation as the fundamental process of the retting of flax.

Behrens (1902) studied the condition favorable for retting of hemp and the organisms concerned. He found that best results were obtained when the amount of hemp was small compared to the volume of solution and when lime was present to neutralize any acids formed. Retting by pure cultures was observed only upon the exclusion of air. The active retting agent he believed to be an anaerobe of the type of *Bacillus amylobacter*

found in soil. Commonly associated wtih this anaerobe was an aerobic organism, *Bacillus asterosporus*. He concluded the retting loosened the plant fibers by dissolving the natural pectin. Störmer (1904) secured similar results with flax, but found the organism to be a plectridium. The products of the pectin decomposition were H_2, CO_2, and certain organic acids. The acids consisted principally of acetic and butyric, with a small amount of valeric and lactic. Beijerinck and Van Delden (1904) isolated certain bacteria which were active in the retting of hemp. These bacteria were shown to attack pectose (protopectin) by means of the enzyme pectosinase. The fermentation of the natural pectin of hemp resulted in the formation of pentose sugars, butyric acid, H_2, and CO_2. They named the organisms producing this enzyme *Granulobacter pectinovorum* and *G. urocephalum*. They were described as anaerobes of the clostridium type. They readily fermented starch, inulin, mannitol, erythritol, glycerol, and gum arabic in addition to the simple sugars.

In the more recently developed methods of retting extensive use has been made of pure cultures of bacteria. The Rossi (1916) process involves the use of *Bacillus comesii*, together with the microorganisms naturally occurring in the material to be retted. This organism is an aerobe similar to *Bacillus asterosporus* described by Meyer (1897). The Carbone process is based on the use of a retting organism in pure culture with an incubation temperature of 35°-39° C. The organism *Bacillus (Clostridium) felsineus* (1922) is an anaerobe. It produces a distinct digestion of potato. In retting, this organism is grown with *Saccharomyces ellipsoideus*.

Another phase of pectin fermentation of practical significance has been studied by those interested in the spoilage of vegetables. One of the first studies in this field was made by Jones (1901) in a study of *Erwinia carotovora*, the cause of soft rot of carrots and other root crops. This led to numerous investigations on the rotting of vegetables. The pectolytic enzyme was studied in detail. It was found to destroy the middle lamella of carrots, causing the cells to separate. The action of pectinase was favored by a temperature of 40°-45° C. and the presence of small amounts of acid. However, the strong acidity of certain plant juices was sufficient to retard its action. A two percent solution of alkali also inhibited the action of pectinase. It acted vigorously on pectin, but not on true cellulose or hemicellulose. It was suggested by Jones that many organisms producing a similar softening of fleshy roots produce pectinase. Harding and Stewart (1902) studied softening of plant tissues by *Erwinia carotovora*, *Pseudomonas campestris* and other organisms. Jones (1905) suggested the only logical means of classifying these organisms was on the basis of the enzymes produced. The organisms causing the soft rots of vegetables are in general pectin fermenters. They grow best at about 30° C. and are gram negative non-spore bearing rods.

Harter and Weimer (1921) compared the production of pectinase of different species of *Rhizopus* with the softening which they produced in sweet potatoes and found marked variations. Pectinase appeared to be an extra-cellular enzyme.

Earlier work concerning the presence, amount and location of pectin in plant tissue was carried out largely by the use of certain dyes and chemical reagents. Such investigators as Mangin (1893), Van Wisselingh (1898), and Tschirch (1908) have been active in this field of work and are

largely responsible for the methods of procedure which are followed in this kind of investigation. Probably the most important reagent for this work is the ruthenium red dye which colors the pectin in the tissues red. The disadvantage, however, in its use is that similar staining reactions are given by lignin, glycogen, and isolichenin. Consequently, many investigators, particularly in recent years, have made quantitative determinations of the pectin content of plant tissue by means of extraction methods. This procedure can be considered as giving approximate results only, since the yield of pectin varies with the method of extraction.

Confusion arising from different methods of extraction, the extent of purification, and the source of the pectin has rendered difficult the determination of its chemical structure. Earlier investigators recorded its physical and chemical properties. A few of these workers such as Frémy (1848), Payen (1856) and later Carré (1922) considered the pectin to be present in the plant as a calcium salt of pectic acid. Tollens (1895) in a study of the composition of pectins from different sources showed that it contained acid radicals. Ehrlich (1917) recognized d-galacturonic acid to be an important constituent of the pectin obtained from many different fruits and plant tissues. He also showed galactose and arabinose to be contained in pectin and concluded the natural pectin to be a calcium-magnesium salt of a complex anhydro-arabino-galacto-methoxy-tetralacturonic acid.

Von Fellenberg (1918) considered the natural pectin (pectose, protopectin) to be a completely methylated ester of pectic acid. He came to this conclusion after he was able to saponify the pectose with the liberation of methyl alcohol. He further concluded that methoxyl groups were present in the pectin molecule. He observed variations in the pectins obtained from different fruits and concluded that the pectin content of older tissue decreased as the lignin content increased. Correns (1921) in work on the natural pectin of flax showed that the solubility of the pectin decreased with the increased methoxyl content. Ritter and Fleck (1923), and Ritter (1925) in studies made of spring and summer woods observed the increase in lignin in the summer wood at the expense of the pectin. F. Ehrlich (1927) also recognized this relationship between pectin and lignin. He considered that pectin might be transformed into lignin during the maturing of the plant. Sucharipa (1924) studied the relation of pectin to the cellulose in plant tissue. He obtained the pectin from lemon peel. After removing all soluble pectin and cellulose by suitable reagents, he demonstrated that additional pectin and cellulose could be developed by hydrolysis. He concluded from this work that pectin and cellulose are intimately combined when occurring naturally in the plant.

Recent progress in our knowledge of the chemistry of pectin has resulted in several proposed structures for the pectin molecule. Ahmann and Hooker (1925) after studying the hydrolytic products of pectic acid propose the following formula for the Bausteine of this compound:

$$(COOH)O_3H_7C_5 < \begin{matrix} O \\ O \end{matrix} > C_5H_9O_3(COOH)$$ (Galacto-galactonic acid.)

Pectic acid, they state, contains at least six of these groups and probably eleven. These are linked together in the form of a ring. Pectin would be represented by covering the carboxyl groups with methyl groups. The chemical structures suggested by Nanji, Paton, and Ling (1925) and as

reported by Dore (1926) postulates a hexa-ring structure for pectin. Four sides of the hexagon nucleus are made up of d-galacturonic acid groups. The fifth and sixth sides consist respectively of a molecule of galactose and one of arabinose. In a demethylated pectic acid this provides four carboxyl groups. Norris and Schyver (1925) have accepted this structure and conclude that in the natural pectin the hydrogen of three of the carboxyl groups only are replaced by methyl groups. The remaining carboxyl may unite with a base or other substance in the plant. Ehrlich (1927) studied natural pectin from sugar beets and was able to show d-galacturonic acid to be the nucleus of the pectin molecule. By producing partial hydrolysis of the protopectin, Ehrlich was able to prepare several poly-galacturonic acids. He concluded that tetra-galacturonic acid constitutes a large part of the pectin molecule, which in addition contains a molecule of galactose and one of arabinose. The natural pectin in the beet was hydrolyzed to a pectic acid, which was conceived as a tri-acetyl-arabino-galacto-dimethoxy-tetragalacturonic acid.

The work of many investigators thus seems to have demonstrated several possibilities as to the composition of pectin and its combination with other substances in plant tissue. Its composition has been shown to vary with different plants, in the different parts of the same plant, and with the stage of growth. Since these qualifications are necessary concerning the pectin present in plant tissue the use of such a tissue for the identification or isolation of pectin-fermenting bacteria may prove to be unreliable. An organism found to produce retting or maceration in plant tissue may not necessarily be capable of fermenting pectin. On the other hand, the organism capable of fermenting pectin may be incapable of producing retting or maceration of plant tissue.

Earlier investigations have emphasized the importance of pectin-fermenting bacteria largely in relation to the retting of fiber plants and to the rotting of vegetables. However, Kruse (1910) refers to many investigators who have designated such common organisms as *Bacillus subtilis, Bacterium coli,* and others as pectin-fermenters. Behrens (1902) had previously attempted to cultivate pectin-fermenting bacteria from retting hemp by use of an extract from the middle lamella as a medium.

More recently investigations of the pectin-fermenting bacteria have been made as to the systematic relationships, their distribution in nature, and the chemical changes they induce in pectin. Due to the increasing interest in this field of work and because of the improved methods in the preparation of pectin, this substance is finding use as a substrate in the study of many different groups of bacteria. Makrinow (1915) used pectin in the study of a soil organism which he named *Pectinobacter amylophilum.* It is a spore former, motile in young cultures, and produces active destruction of potato with gas formation. It is active in the fermentation of starch as well as pectin. Orla-Jensen (1919) in a scheme of classification has designated *Pectobacillus* as a genus for the clostridial and plectridial species of bacteria capable of fermenting pectin. Düggeli (1921) studied the prevalence of pectin-fermenting bacteria in the soil. In a table containing the results of this study included in Waksman's (1927) "Principles of Soil Microbiology," is given the number of bacteria as determined in different types of soil. Deciduous forest and marsh land soil show the lowest number of pectin-decomposing bacteria, while the largest number may be found in

garden soil and in coniferous forest soil. Kluyver and Donker (1926) in a treatise on the fermentative processes of certain facultative anaerobic bacteria, refer to the use of lemon pectin. This pectin was reported as pure and was used as a 2% solution. Their strains 11 and 13 gave a distinct fermentation on this pectin medium. As the result all alcohol precipitatable material was fermented. Coles (1926) employed a lemon pectin in an attempt to develop a differential medium for the colon-typhoid group of bacteria. In this study a number of strains of *Bacterium coli, Bact. aerogenes, Bact. oxytocum, Bact. cloacae, Bact. viscosum-aerogenes,* and *Bacillus aceto-ethylicus* were used. He found that pectin was fermented with the production of acid and gas by 7 out of 13 strains of *Bact. oxytocum,* 4 of 9 strains of *Bact. aerogenes,* 2 strains of *Bact. viscosum-aerogenes,* and by 2 strains of *Bacillus aceto-ethylicum.* No description was given of the pectin used. Weyer and Rettger (1927) in an extensive study of butyl alcohol and acetone-producing bacteria, designate the different strains of *Clostridium aceto-butylicum* as pectin-fermenting bacteria. No mention was made of the source of the pectin employed for this study.

The investigations having to do with pectin and its fermentation by micro-organisms have been conducted under such varied conditions as largely to prevent a comparison of the results. In many cases naturally occurring pectin was employed, which has been secured from different plants. The usual custom was to use plant tissue. In many reports no mention is made of the kind or character of the pectin used. As a result, there is much confusion in the literature regarding the question of retting, and the organisms responsible for this process. Furthermore, there has not been a clear understanding of the organisms causing soft rots of vegetables and as a result no adequate scheme for the classification of these organisms has been provided. Our knowledge concerning the pectin fermenting bacteria is limited and fragmentary.

EXPERIMENTAL

This study had for its purpose the isolation and study of organisms capable of fermenting pectin. The organisms were isolated from several different sources. It was hoped to gain a fair idea of the occurrence of pectin-fermenting bacteria in nature. A study of certain organisms which have been found to ferment pectin by other investigators was also undertaken.

METHODS FOR THE ISOLATION AND STUDY OF BACTERIA FERMENTING PECTIN.

This part of the work consisted in the isolation of organisms for many different sources, and of a study of their morphology, their cultural characteristics and their fermentation reactions on many different sugars, alcohols, and poly-saccharides, including pectin.

Methods and technique. The pectin used was supplied by the California Fruit Growers' Exchange. It is a very fine, nearly white powder. It forms an opalescent solution in water, and exhibits colloidal properties. An attempt to filter the solution through an Empire bacteriological filter by suction was abandoned as filtration occurred very slowly. The specific

rotation was $(\alpha) = +185.5°$ at 30° C. This reading was obtained from a 0.5% pectin solution in water. The cylinder used in the examination of the solution was 10 cm. long. The pectin solution contained no reducing substances when tested with Fehling's solution.

In a communication from Dr. C. P. Wilson, Director of the research laboratories of the California Fruit Growers' Exchange, the method of preparation is given as follows: "The pectin sent you was prepared in accordance with U. S. Patent No. 1,497,884. The method of preparation is briefly as follows: Chopped lemon peel from which citric acid has been removed is extracted with a 0.5% solution of sulphurous acid for about an hour at 90° C. The acid solution containing the pectin is drawn off and cooled and the pectin precipitated by means of aluminum hydroxide, which is formed in the solution by adding in rapid succession and with violent agitation sufficient NH_4OH and $Al_2(SO_4)_3$ to precipitate the pectin. The amounts of reagents are determined by laboratory tests.

"The precipitated pectin is drained of its mother liquor, washed with water, dried and ground. The dry powdered product is suspended in strong alcohol containing enough HCl to convert the aluminum hydroxide present into aluminum chloride, which being soluble in alcohol is removed when the latter is drawn off. After further washing with neutral alcohol the product is dried, when it is reground and is then ready for use. The ash content of the finished pectin is from about 1.5 to 3%, most of which is probably Al_2O_3.

"The product which you have been using was made in accordance with the above method and from the many statements made to us by practical users as well as by persons doing research work on pectin, it appears that this is the purest pectin which has become available commercially."

These facts concerning the properties and preparation of pectin seem to warrant its use as a dependable substrate. The medium commonly employed consisted of 0.3% pectin, 0.2% K_2HPO_4, and 0.2% NH_4Cl in water. It was adjusted to pH 7.0-7.2, tubed in Durham tubes, and sterilized in the autoclave at 121° C. for 15 minutes. Pectin agar consisted of the same constituents with an addition of 1.5% agar. Since the pectin medium contains no reducing substances or other fermentable material other than pectin, any fermentation must be due to the pectin itself.

The first method of procedure was to inoculate a tube of the medium with any substance suspected of containing pectin fermenters, if fermentation occurred, then a transfer was made from this tube to a second tube and frequently to a third tube before the fermenting material was finally plated out for the isolation. Later work indicated that whenever fermentation occurred in the first tube, it could be plated and the organisms isolated without enrichment. The fermenting mixture was plated on pectin agar. The colonies that grew were cultured and again inoculated into pectin Durham tubes. In case fermentation (acid and gas formation) occurred, the fermenting mixture was plated on glucose-phosphate agar to detect any possible contamination. Whenever a mixture was present in the last plating, the cultured colonies were again returned to the pectin medium and replated. In no case was any organism recorded as a pectin fermenter unless it produced *both acid and gas* from pectin.

The organisms studied were isolated from the following sources: A soil preparation, creek water, decayed potatoes and parsnips, cornstalks,

insect contamination, hay infusions, and sewage. The prepared soil consisted of a mixture of garden, cornfield and residual forest soils with a frequent application of ground cornstalks. This mixture of soil was treated from time to time with phosphate, lime and ammonium salts. It was kept in good tilth by frequent cultivations and by preserving an optimum moisture content. This soil was an important source of organisms throughout the work. A further study of different sources revealed that pectin-fermenting bacteria were present in practically all decayed material as well as in certain spoiled canned vegetables. The canned vegetables studied consisted of tomatoes, Swiss chard, peas, beans, spinach, asparagus, and pumpkin. It was an interesting observation that wherever pectin-fermenting organisms were found in canned material the vegetable contents showed a softened condition and frequently the production of gas.

The preparation of the different media containing sugars, polysaccharides, alcohols, and glucosides was carried out with the utmost care. Each medium in addition to the carbohydrate to be studied consisted of 1% peptone, 0.2% K_2HPO_4, and Andrade indicator. The carbohydrate was used in concentrations ranging from 0.3% to 0.5%. The solutions were adjusted to pH 7.0, tubed in Durham tubes, and sterilized in the autoclave at 121° C. for 12-15 minutes. This method of sterilization was saitsfactory in most cases since the tubes were rapidly cooled after leaving the autoclave. The different solutions were tested by means of bacteria of known fermentative powers to determine whether the sugars had been broken down by sterilization. In only a few cases was there any indication of such a change. In later work the carbohydrate was sterilized separately and added to the medium under sterile conditions. To facilitate this a 2% solution was sterilized and added in correct amount by means of a sterile pipette to the Durham tubes containing the basic medium. Ample incubation was allowed before inoculation in order to eliminate any contaminated tubes and to permit dispersion of the carbohydrate.

Indol production was determined by growing the cultures in tryptophane broth for 3-5 days. The Ehrlich test was employed. A few drops of the para-di-methyl-amino-benzaldehyde solution, prepared according to standard methods, was placed on the lower tip of the cotton stopper along with a drop or two of concentrated HCl. The cotton stopper was again placed in the tube and forced down to within 1½ inches of the liquid. The tubes were then placed in boiling water to facilitate volatilization of the indol. A distinct pink coloration of the cotton stopper indicated the presence of the indol. The tubes were always boiled, but observation showed this was unnecessary with most of the cultures since sufficient indol had collected in the cotton stopper to produce the pink condition as soon as the reagents were added.

Proteolysis of gelatin was determined by means of the Frazier (1926) test. This consisted in growing a giant colony in the center of a Petri dish on medium containing phosphates (0.1%) and gelatin (0.1%) in addition to 1.5% agar and 0.1% peptone. The test was carried out by the use of two such plates. After several days' growth, one plate was flooded with 1% tannic acid solution and the other plate was flooded with acidified 0.2% mercuric chloride solution. Tannic acid solution precipitates the proteoses, but has no effect on amino acids or simpler nitrogen compounds. In case of porteolysis, a clear zone surrounds the colony and beyond this is a white

precipitated band. Where there is no proteolysis the plate is of uniformly white appearance. The mercuric chloride solution precipitates the proteins, but has no effect on peptones, proteoses, or simple nitrogen compounds. A plate showing proteolysis when flooded with the acidified HgCl shows a clear zone about the colony. The remainder of the plate wheer the proteolytic enzymes had not reached appears opaque white. The ordinary gelatin liquefaction tube method was used as a control on the cultures which gave a positive proteolysis test.

The organisms were also grown on sterile slices of Irish potatoes, sweet potatoes, carrots, parsnips, and apples. This study was carried out on the assumption that pectin fermenting bacteria would soften such vegetables. The vegetables were prepared in the following manner. Large specimens were thoroughly washed in water and then in a 60% alcohol solution. The top and bottom sides, which would come in contact with the cutting apparatus, were sliced off by means of a sterilized scalpel. Cylinders were now cut out by means of sterilized apple borer and dropped into a pan of sterile boiling water. After a few seconds they were removed, cut to form a slanting surface and introduced into sterilized potato tubes. The tubes were immediately placed in an Arnold sterilizer and heated at 75° C. for one hour. This sterilizing procedure was used on the two following days. Sufficient incubation was allowed to insure the use of sterile vegetables.

It has been commonly reported that pectin fermenters are usually active starch digesters. Consequently, additional study was made of this character, besides the use of "soluble starch" in the Durham fermentation tubes. Starch agar was prepared by adding a sterilized solution of starch to a bottle of melted nutrient agar so as to make a medium of 0.2% starch. The agar was poured into plates and allowed to solidify. In this manner starch agar plates were prepared with both "soluble starch" and commercial corn starch. The culture was streaked over the plates, following the technique used by Allen (1918). After two days' incubation at 37° C. the plates were flooded with a saturated iodine solution in 50% alcohol. Diastatic action was indicated by a wide uncolored area about the colonies.

Pectin-fermenting bacteria have been considered significant because of their ability to ret flax and hemp. Since this investigation was carried out in connection with studies on the fermentation of cornstalks it was important to know whether these pectin-fermenting bacteria would ret cornstalks. The latter were cut in slices by means of a sharp knife. These slices were sterilized in the autoclave at 121° C. for an hour. After sterilization a nutrient solution was added containing 0.1% K_2HPO_4, 0.1% NH_4Cl, and 0.2% pectin. The tubes of these sliced cornstalks were then inoculated with the culture to be tested.

The production of acetyl-methyl-carbinol was determined for all of the organisms studied by making a qualitative test of certain of the sugar fermentation cultures. The cultures employed were those growing on glucose, sucrose, mannitol and salicin broth medium. The test was made after fermentation had proceeded for 36 to 48 hours by adding 10% KOH in amount equal to the volume of the culture. It was then gently agitated and incubated at 37° C. Observations were made after three, twelve and twenty-four hours. Motility tests were made from 18 to 24 hours old broth cultures by the hanging drop method. These results were checked by means of stab cultures in semi-solid agar. Capsule formation was

studied from 24 hour old cultures in litmus milk. Capsule formation was determined by Welch's glacial acetic acid method.

The non-sporebearing pectin-fermenting bacteria showed a very close resemblance to certain species of the genera *Escherichia* and *Aerobacter* and therefore some study was made with reference to their sanitary significance. This study was made by streaking the cultures on plates of Endo and Eosin-methylene-blue media. The colonies of these organisms were compared to the colonies of *Escherichia coli* and *Aerabacter aerogenes* under the same conditions after both a two and a four day incubation period.

GENERAL CHARACTERISTICS OF THE PECTIN-FERMENTING BACTERIA

The pectin-fermenting organisms isolated were found to be included in the genera *Aerobacter, Bacillus,* and *Clostridium.*

The organisms fermenting pectin belonging to the genus *Aerobacter* are rather small gram negative rods without spores, frequently showing bipolar staining and manifesting exceptional ability in the fermentation of a large number of sugars, polysaccharides, alcohols, and glucosides. They are further characterized by rarely exhibiting any protolytic action and by the production of acid and gas in litmus milk without any curd formation. These organisms are usually very strong indol and acetyl-methyl-carbinol producers. They agree with the characteristics of the genus *Aerobacter* in ability to oxidize the acids formed from many of the sugars, and consequently are methyl-red negative. But unlike many of the genus *Aerobacter* they are very active pectin fermenters, producing acid and gas and showing the ability to soften vegetable tissue.

The sporulating aerobic pectin-fermenters are very similar to *Bacillus aceto-ethylicum.* They are gram negative, very long, thin rods. They are motile but unlike *B. aceto-ethylicum* they liquefy gelatin. They are likewise similar to this organism in producing the same characteristic slow growth on all artificial media. However, they are very active fermenters, producing acid and gas from all sugars, polysaccharides, and glucosides employed, but fail to ferment a few of the polyatomic alcohols. Litmus milk is fermented with acid and gas production. Pectin is also fermented with the formation of acid and gas. Pentosan prepared from cornstalks was also fermented with acid and gas production. Potatoes, carrots, and apples are rapidly macerated by this group of organisms. As a result of this maceration all of the tissue is destroyed with the exception of the tracheal tubes. Indol and acetyl-methyl-carbinol are not produced. These organisms in addition show the ability to ret cornstalks slowly, so that the fibrous parts are easily separated from the pith.

The group of anaerobic pectin fermenters includes a large number, many of which seem to be better classified as micro-aerophiles than as true anaerobes. These organisms are generally gram positive, motile, and form spores, but they rarely appear as true clostridia. They are active fermenters, but rarely attack the alcohols. The colony is compact and of light brownish color. Usually there is no (or very slow) liquefaction of gelatin Pectin is fermented with the formation of acid and gas. Some cultures are also active pentosan fermenters. These organisms also soften vegetables, especially potatoes and carrots.

STUDIES ON THE PECTIN FERMENTING BACTERIA BELONGING TO THE GENUS *AEROBACTER*

The results of the detailed study of these organisms are given in Table I.

These results show the close relationship existing between many aerobic non-sporeforming pectin-fermenting bacteria obtained from a variety of sources. These organisms are characterized by many of the same features reported for the spore-forming, pectin-fermenting bacteria by previous investigators. Proteolysis is absent or very slow; there is active fermentation of many carbohydrates, including pentose sugars and starch; the production of a softened condition in vegetable tissue is a characteristic of most of these organisms. Another character possessed by these organisms is the ability to oxidize the acids formed. In certain of the sugars and alcohols, such as levulose, mannose, galactose, xylose, arabinose, dulcitol, and sorbitol, the organisms rapidly reduce the acidity. In other carbohydrates this characteristic is variable. With glycerol, mannitol, adonitol, trehalose, sucrose, and lactose utilization of the acids formed is very rare. A study of the ability to reverse the reaction often was an aid to the classification of the organisms.

The production of both indol and acetyl-methyl-carbinol is a prominent characteristic. This is true for all but a very few of the forms isolated. A few organisms produce acetyl-methyl-carbinol without the formation of indol.

The fermentation of pectin was of such a nature as to destroy all of the alcohol precipitable material. In addition to the formation of acid and gas, all of the cultures produced reducing substances when tested by Fehling's solution.

None of the organisms of this group was able to ret cornstalks.

LEGEND FOR TABLE I

s.r = Short rod
\+ = Positive reaction
− = Negative reaction
1 2 3, 4 = Proportional reaction
+r = Acidity followed by reduction
Sl. = Slight
s = Slow
? = Questionable
* = Ring
§ = Sediment
Pel. = Pellicle
F = Flaky
L. Brown = Light brown
D. Green = Dark green
A = Acid
G = Gas
S = Softened
R = Reduction
C = Curd
Cl = Cloudy
Gl = Glucose
Su = Sucrose
Sa = Salicin
M = Mannitol

TABLE I. CULTURAL CHARACTERISTICS OF PECTIN-FERMENTING BACTERIA OF THE GENUS AEROBACTER.

Culture No.	Shape	Size in μ		Arrangement	Gram Reaction	Granules		Capsules
		Wide	Long			Iodine	M.B.	
1	s.r	0.8-1	1.2-4.5	Single	—	+	+	1
2	s.r	0.8-1	0.8-4.5	Single	—	+	—	2
3	s.r	0.8-1.0	1.5-3.8	Single	—	+	+	1
4	s.r	0.6-0.8	2.0-4.5	Single	—	+	+	2
5	s.r	0.8-1	1.5-3.0	Single	—	+	—	1
6	s.r	0.8-1.2	1.2-3.5	Single	—	?	—	2
7	s.r	1.0-1.2	1.5-5.0	Single	—	+	+	3
8	s.r	1.0-1.2	1.5-3.5	Single	—	+	—	3
9	s.r	1.0-1.2	1.5-4.0	Single	—	+	—	3
10	s.r	1.0-1.2	2.0-4.5	Single	—	+	+	2
11	s.r	1.0-1.2	0.8-3.0	Single	—	?	—	1
12	s.r	1.0-1.5	1.5-3.0	Single	—	+	+	2
13	s.r	0.8-1.0	2.0-5.0	Single	—	+	+	2
14	s.r	1.0-1.2	1.5-3.0	Single	—	+	—	2
15	s.r	1.0-1.2	2.0-5.0	Single	—	+	—	2
16	s.r	1.0-1.2	1.7-2.5	Single	—	+	—	2
17	s.r	0.6-0.8	0.8-2.0	Single Chain	—	+	—	2
18	s.r	0.6-0.8	1.5-3.5	Single Chain	—	+	—	1
20	s.r	0.7-1.0	2.0-4.5	Single	—	+		3
21	s.r	1.0-1.2	2.0-5.0	Single	—	+	+	3
22	s.r	0.9-1.0	2.5-4.5	Single	—	+	+	3
23	s.r	0.6-0.8	0.8-2.0	Single Chain	—	+	—	1
24	s.r	1.0-1.2	1.5-3.0	Single Chain	—	+	+	1
25	s.r	0.3-0.5	0.4-1.5	Single Chain	—	+	—	1
26	s.r	0.7-1.0	1.6-2.0	Single	—	+	+	2
27	s.r	1.0-1.2	1.8-3.0	Single	—	+	+	2
28	s.r	0.8-1.0	1.5-3.5	Single	—	+	+	1
29	s.r	0.8-1.0	1.5-3.5	Single	—		+	1
30	s.r	0.6-0.8	0.8-2.0	Single	—		—	2
31	s.r	0.8-1.0	0.8-3.0	Single	—		+	2
32	s.r	1.0-1.2	2.0-4.5	Single	—		—	2
33	s.r	0.9-1.2	1.5-3.0	Single	—		+	1
34	s.r	0.8-1.0	1.5-3.0	Single	—		+	2
35	s.r	0.8-1.0	1.5-3.0	Single	—		—	3
36	s.r	0.6-0.8	1.0-3.0	Single	—		—	—
37	s.r	0.6-0.8	1.0-1.2	Single	—		—	—
38	s.r	0.8-1.0	1.0-2.5	Single	—	2	—	2
39	s.r	0.6-1.0	1.2-3.0	Single	—	2	+	2

TABLE I—(Continued)

Culture No.	Spores	Motility	Indol	Acetyl-Methyl-Carbinol				Nitrate reductions	Uric Acid	M.R. reaction
				Gl	Su	M	Sa			
1	—	—	+	+	+	—	—	+	3	—
2	—	—	+	+	+	—	+	+	3	—
3	—	—	+	+	+	+	—	+	3	—
4	—	—	+	+	—	+	—	+	3	—
5	—	—	+	+	+	?	?	+	2	—
6	—	—	+	+	+	?	?	+	2	—
7	—	—	+	+	+	—	?	+	3	—
8	—	—	+	—	—	?	—	+	3	—
9	—	—	+	+	+	—	+	+	3	—
10	—	—	+	+	+	—	—	+	3	—
11	—	—	+	+	+	+	—	+	3	—
12	—	—	+	+	+	?	?	+	3	—
13	—	—	+	+	+	?	?	+	3	—
14	—	—	+	+	+	—	—	+	2	—
15	—	—	+	+	+	—	—	+	3	—
16	—	—	+	+	+	—	—	+	3	—
17	—	+	—	—	—	—	+	+	1	—
18	—	+	—	+	+	—	+	+	2	—
20	—	—	+	+	+	—	sl	+	3	—
21	—	—	+	+	+	+	—	+	2	—
22	—	—	+	+	+	+	—	+	3	—
23	—	+	+	+	+	+	—	+	3	—
24	—	—	+	+	+	—	—	+	3	—
25	—	+	—	+	+	+	—	+	3	—
26	—	—	+	+	+	+	?	+	3	—
27	—	—	+	+	+	?	?	+	3	—
28	—	—	+	+	+	?	?	+	3	—
29	—	—	+	+	+	?	?	+	3	—
30	—	—	+	+	+	+	—	+	2	—
31	—	—	+	+	+	+	—	+	2	—
32	—	—	+	+	+	—	?	+	3	—
33	—	—	+	+	+	—	+	+	3	—
34	—	—	—	+	+	—	+	+	3	—
35	—	—	+	+	+	—	—	+	2	—
36	—	+	—	+	+	—	—	+	2	—
37	—	+	—	+	+	—	—	+	3	—
38	—	—	+	+	+	?	—	+	2	—
39	—	—	+	+	+	—	—	+	2	—

TABLE I—(Continued)

Culture No	H_2S	Proteolysis	Skatol	Source	Broth	Litmus Milk A	Litmus Milk G	Litmus Milk R	Litmus Milk C	Diastase
1	1	—	—	Insect	Cloudy	+	—	—	—	+
2	1	—	—	Rotted pot.	Cloudy	+	—	—	—	—
3	1	s	—	Rotted pot.	Cloudy§	+	—	—	—	—
4	2	—	—	Hay infus.	F. Pel.§	+	—	—	—	—
5	2	—	—	Mixed cult.	Cloudy	+	+	—	—	—
6	2	s	+	Mixed cult.	Cloudy	+	+	—	—	+
7	1	—	—	Spec. soil	Cloudy	+	+	—	—	—
8	1	—	—	Spec. soil	Cloudy	+	+	—	—	+
9	1	—	—	Spec. soil	Cloudy	+	+	—	—	—
10	3	—	—	Spec. soil	Cloudy	+	+	—	—	+
11	4	—	—	Spec. soil	Cloudy	+	+	—	—	—
12	2	—	—	Creek water	Cloudy	+	+	—	—	+
13	2	—	—	Creek water	Cloudy	+	+	—	—	+
14	1	—	—	Creek water	Cloudy	+	—	—	—	+
15	1	—	+	Creek water	Cloudy	+	+	—	—	—
16	1	—	—	Creek water	Cloudy	+	+	—	—	+
17	1	—	—	Creek water	Cl. Pel.	+	+	—	—	—
18	1	—	—	Creek water	Cl. Pel.	+	+	+	—	—
20	1	—	—	Creek water	Cloudy*	+	+	—	—	+
21	2	s	?	Creek water	Cloudy	+	+	—	—	—
22	1	s	—	Creek water	Cloudy	+	+	—	—	—
23	2	—	+	Rotted pot.	Cloudy*	+	—	+	+	—
24	2	—	+	Rotted pot.	Pel.	+	+	—	—	+
25	2	s	—	Rotted pot.	Cloudy*	+	+	+	+	—
26	1	—	+	Mixed cult.	Cloudy	+	+	—	—	—
27	1	—	+	Mixed cult.	Cloudy	+	+	—	—	—
28	2	—	—	Creek water	Cloudy	+	+	s	—	+
29	2	—	—	Creek water	Cloudy	+	+	s	—	+
30	1	—	+	Hay infus.	Cloudy§	+	+	—	+	—
31	1	—	+	Hay infus.	Cloudy§	+	+	—	+	—
32	1	—	+	Creek water	Cloudy	+	+	—	+	+
33	3	—	+	Creek water	Cloudy*	+	+	s	+	+
34	?	—	—	Hay infus.	Cloudy	+	+	—	+	—
35	?	—	—	Hay infus.	Cloudy	+	+	—	+	—
36	2	+		Rotted pot.	Cloudy*	+	?	—	+	+
37	1	+		Rotted pot.	Cloudy*	+	+	—	+	—
38	1	—	—	Hay infus.	Cloudy*	+	+	—	+	—
39	2	—	+	Creek water	Cloudy	+	+	—	—	—

TABLE I—(Continued)

Culture	Glucose		Levulose		Mannose		Galactose		Maltose		Sucrose		Lactose	
No.	A	G	A	G	A	G	A	G	A	G	A	G	A	G
1	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	+
2	+	+	+r	+	+	+	+r	+	+r	+	+	+	+	+
3	+	+	+r	+	+r	+	+	+	+	+	+	+	+r	+
4	+	+	+r	+	+	+	+r	+	+r	+	+	+	+	+
5	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	+
6	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	+
7	+	+	+	+	+r	+	+	+	+	+	+	+	+r	+
8	+	+	+	+	+r	+	—	—	+	+	+	+	+r	+
9	+	+	+	+	+	+	+r	+	+	+	+	+	+r	+
10	+	+	+r	+	+r	+	+	+	+r	+	+	+	+	sl
11	+	+	+	+	+	+	+r	+	+	+	+	+	+r	+
12	+	+	+	+	+r	+	+	+	+	+	+	+	+	+
13	+	+	+r	+	+r	+	+r	+	+	+	+r	+	+r	+
14	+	+	+	+	+	+	+r	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+r	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	sl
18	+r	+	+r	+	+r	+	+r	+	+r	+	+r	+	+	+
20	+	+	+	+	+r	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+r	+	+r	+	+	+	+	+	+	+
22	+	+	+	+	+r	+	+r	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+r	+	+	+	+	+	+	+
24	+	+	+r	+	+r	+	+r	+	+r	+	+	+	+	+
25	+	+	+r	+	+r	+	+r	+	+r	+	+	+	+	+
26	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	+
27	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	+
28	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	+
29	+	+	+r	+	+r	+	+r	+	+	+	+	+	+	+
30	+	+	+r	+	+r	+	+r	+	+r	+	+	+	+	+
31	+	+	+r	+	+r	+	+r	+	+r	+	+	+	+	+
32	+	+	+	+	+	+	+r	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+r	+	+r	+	+	+	+	+
34	+	+	+r	+	+r	+	+r	+	+r	+	+	+	+	+
35	+	+	+	+	+r	+	+	+	+r	+	+	+	+	+
36	+	+	+r	+	+r	+	+r	+	+r	+	+	+	+	+
37	+r	+	+r	+	+r	+	+r	+	+r	+	+r	+	+	+
38	+	+	+r	+	+r	+	+	+	+	+	+	+	+	+
39	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE I—(Continued)

Culture No.	Raffinose A	Raffinose G	Rhamnose A	Rhamnose G	Trehalose A	Trehalose G	Melezitose A	Melezitose G	Salicin A	Salicin G	Amygdalin A	Amygdalin G	Aesculin A	Aesculin G	Xylose A	Xylose G
1	+	+	+	+	+	+r	—	—	+	+	—	—	+	+	+r	+
2	+r	+	?	?	+	+	—	—	+r	+	—	—	+	+	+	+
3	+	+	+	—	+	+	—	—	+	+		—	+	+	+	+
4	+	+	+	—	+	+	—	—	+	+	?	—	+	+	+r	+
5	+	+	+	+	+	+	—	—	+	+	?	—	+	+	+	+
6	+	+	+	+	+	+	—	—	+	+	?	—	+	+	+	+
7	+	+	+	?	+	+	+r	+	+	+	?	—	+	+	+	+
8	+	+	+	?	+	+	+r	+	+	+	?	—	+	+	+	+
9	+	+	+	?	+	+	+r	+	+	+	—	—	+	+	+	+
10	+	+	+	?	+	+	+r	+	+	+	?	—	+	+	+r	+
11	+	+	+	?	+	+	+r	+	+	+	?	—	+	+	+	+
12	+	+	+	?	+	+	—	—	+	+	?	?	+	+	+	+
13	+r	+	+	?	+	+	—	—	+	+	?	—	+	+	+r	+
14	+	+	+	+	+	+	—	—	+	+	?	—	+	+	+r	+
15	+	+	+	+	+	+	—	—	+	+	?	—	+	+	+r	+
16	+	+	+	?	+	+	—	—	+	+	?	—	+	+	+r	+
17	—	—	+	—	+r	+	—	—	+	+	?	—	+	+	+r	+
18	—	—	—	—	+r	+	—	—	+	+	—	—	+	+	+	+
20	+	+	+	+	+	+	—	—	+	+	?	—	+	+	+r	+
21	+	+	+	+	+	+	—	—	+	+	—	—	+	+	+	+
22	+	+	+	+	+	+	—	—	+	+	?	—	+	+	+r	+
23	+r	+	+r	+	+r	+	—	—	+r	+	—	—	+	+	+	+
24	+r	+	+r	+	+r	+	—	—	+	+	—	—	+	+	+r	+
25	+r	+	sl	sl	+r	+	—	—	+r	+	—	—	+	+	+	+
26	+	+	+	+	+	+	—	—	+	+	—	—	+	+	+	+
27	+	+	+	+	+	+	—	—	+	+	—	—	+	+	+	+
28	+	+	+	?	+	+	—	—	+	+	—	—	+	+	+r	+
29	+	+	+	?	+r	+	—	—	+	+	—	—	+	+	+r	+
30	+	+	+r	+	+r	+	—	—	+	+	—	—	+	+	+r	+
31	+	+	+r	+	+r	+	—	—	+	+	—	—	+	+	+r	+
32	+	+	+	+	+	+	—	—	+	+	—	—	+	+	+r	+
33	+	+	+	+	+	+	—	—	+	+	—	—	+	+	+r	+
34	+r	+	+	+	+r	+	—	—	+	+	—	—	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	+
36	+	+	+	?	+r	+	—	—	+	+	—	—	—	—	+r	+
37	+r	+	+	?	+r	sl	—	—	+r	+	—	—	—	—	+r	+
38	+r	+	+r	+	+r	+	+r	+	+	+	—	—	+	+	+	+
39	+	+	+r	+	+	+	—	—	+	+	—	—	+r	+	+	+

TABLE I—(Continued)

Culture	Arabinose		Glycerol		Mannitol		Dulcitol		Sorbitol		Adonitol		Inositol		Erythritol	
No.	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
1	+r	+	+	+	+	+	+r	+	+r	+	+	+	+r	+	—	—
2	+r	+	+	+	+	+	—	—	+r	+	+	+	+	+	—	—
3	+r	+	+	+	+	+	—	—	+r	+	+r	+	+	+	—	—
4	+r	+	+	+	+	+	—	—	+	+	+	sl	?	?	—	—
5	+r	+	+	+	+	+	+r	+	+r	+	+	+	+	+	—	—
6	+r	+	+	+	+	+	+r	+	+r	+	+	+	+	+	—	—
7	+r	+	+	+	+	+	—r	—	+r	+	+	+	+	+	—	—
8	+r	+	+	+	+	+	—	—	+r	+	+	+	+r	+	—	—
9	+r	+	+	+	+	+	—	—	+r	+	+	+	?	—	—	—
10	+r	+	+	+	+	+	—	—	+	+	+	+	+	—	—	—
11	+r	+	+	+	+	+	—	—	sl	sl	+	+	+	+	—	—
12	+r	+	+	+	+	+	+r	+	+r	+	+	+	+	+	—	—
13	+r	+	+r	+	+	+	+r	+	+r	sl	+	+	+	+	—	—
14	+r	+	+	+	+	+	+r	+	+r	sl	+	+	+	+	—	—
15	+r	+	+	+	+	+	+r	+	+r	sl	+	+	+	+	—	—
16	+r	+	+	+	+	+	+	+	sl	sl	+	+	+	—	+	?
17	+r	+	—	—	+	+	—	—	+r	+	—	—	—	—	—	—
18	+r	+	—	—	+	+	—	—	+r	+	—	—	—	—	—	—
20	+r	+	+	+	+	+	+r	+	+r	+	+	+	+	+	—	—
21	+r	+	+	+	+	+	+r	+	+	+	+	+	+	+	—	—
22	+r	+	+	+	+	+	+r	+	+	+	+	+	+	+	—	—
23	+r	+	—	—	+	+	+	+	+r	+	+r	+	+	+	—	—
24	+r	+	+	+	+	+	—	—	+r	+	+r	+	+r	+	—	—
25	+r	+	—	—	+	+	+r	+	+r	+	—	—	—	—	—	—
26	+r	+	+	+	+	+	+r	+	+r	sl	+	+	+	+	—	—
27	+r	+	+	+	+	+	+r	+	+r	sl	+	+	+	+	—	—
28	+r	+	+	+	+	+	+r	+	+r	+	+	+	+	sl	—	—
29	+r	+	+	+	+	+	+r	+	+r	+	+	+	+	sl	—	—
30	+r	+	+	+	+	+	—	—	+r	+	+	+	+	+	—	—
31	+r	+	+	+	+	+	—	—	+r	+	+	+	+	+	—	—
32	+r	+	+r	+	+	+	+	+	+r	+	+	+	+	+	—	—
33	+r	+	+	+	+	+	+r	+	+r	+	+	+	+r	+	+	?
34	+r	+	+	+	+	+	—	—	+r	+	+r	+	+r	+	—	—
35	+r	+	+	+	+	+	+r	+	+r	+	+r	+	+r	+	—	—
36	+r	+	—	—	+	+	—	—	+r	+	—	—	—	—	—	—
37	+r	+	—	—	+	+	—	—	+r	+	—	—	—	—	—	—
38	+r	+	+	+	+	+	+r	+	+r	+	+r	+	+r	+	—	—
39	+r	+	+	+	+	+	+r	+	+r	sl	+	+	+	+	—	—

TABLE I—(Continued)

Culture	Glycogen		Dextrin		Sol. Starch		Inulin		Pectin		Pentosan		Lignin		Cellulose	
No.	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
1	+r	+	+r	+	+r	+	+r	+	+	+	+	—	—	—	—	—
2	+	+	—	—	+r	+	—	—	+r	+	—	—	—	—	—	—
3	+r	+	—	—	+	+	—	—	+r	+	—	—				
4	+r	+	—	—	+	+	—	—	+r	+	—	—				
5	+r	+	+r	+	+r	+	+r	+	+	+	—	—				
6	+r	+	+r	+	+r	+	+	+	+	+	—	—	—	—		
7	+	+	?	—	+	+	—	—	+r	+	—	—	—	—		
8	+r	+	—	—	+r	+	—	—	+r	+	—	—				
9	+r	+	—	—	+r	+	—	—	+r	+	—	—	—	—		
10	+	+	—	—	+	+	—	—	+r	+	—	—				
11	+r	+	—	—	+	+	—	—	+r	+	—	—				
12	+r	+	+r	+	+r	+	+r	+	+r	+	—	—	—	—		
13	+r	+	+r	+	+r	+	+r	+	+r	+	—	—				
14	+r	+	+r	+	+r	+	+	+	+r	+	—	—				
15	+r	+	+	+	+	+	+r	+	+r	+	—	—				
16	+r	+	+r	+	+r	+	+	+	+r	+	—	—				
17	—	—	—	—	—	—	—	—	+r	+	—	—	—	—	—	—
18	+r	sl	—	—	—	—	—	—	+r	+	—	—	—	—	—	—
20	+r	+	+r	+	+	+	+	+	+r	+	—	—				
21	+r	+	—	—	+r	+	+	+	+r	+	—	—				
22	+r	+	+r	+	+	+	+r	+	+r	+	—	—	—	—		
23	+r	+	—	—	—	—	—	—	+r	+	—	—				
24	+r	+	—	—	+	+	—	—	+r	+	—	—			—	—
25	—	—	—	—	—	—	—	—	+r	sl	—	—				
26	+r	+	+r	+	+	+	+r	+	+r	+	—	—				
27	+r	+	+r	+	+	+	+r	+	+r	+	—	—				
28	+r	+	+r	+	+r	+	+r	+	+	+	—	—				
29	+	+	+r	+	+r	+	+r	+	+	+	—	—	—	—		
30	+	sl	—	—	—	—	—	—	+	+	—	—				
31	+	sl	—	—	—	—	—	—	+	+	—	—				
32	+r	+	+	+	+r	+	+r	+	+	+	—	—				
33	+r	+	+r	+	+r	+	+	+	+r	+	—	—			—	—
34	+r	+	—	—	+r	+	—	—	+	sl	—	—				
35	+r	+	—	—	+r	+	—	—	+	+	—	—				
36	—	—	—	—	+	+	—	—	+r	sl	—	—				
37	—	—	—	—	—	—	—	—	+r	sl	—	—				
38	+r	+	—	—	+	+	—	—	+r	+	—	—				
39	+r	+	—	—	+	+	—	+	+r	+	—	—				

TABLE I—(Continued)

Culture	Potato			Carrot			Parsnip			Apple		
No	Color	G	S	Color	G	S	Color	G	S	Color	G	S
1	Dark	—	1	Normal	—	—	Normal	?	1	Normal	?	—
2	Dark	—	+	D. Green	1	1	D. Green	2	1	Normal	2	2
3	Dark	+	1—	D. Green	2	2	Normal	1	4	Normal	2	1
4	Dark	1	1	Sl. Dark	?	?	Normal	1	1	Normal	2	2
5	Normal	1	3	Normal	2	3	Normal	1	?	Normal	2	2
6	Normal	?	2	Normal	—	—	Normal	1	—	Normal	—	—
7	Gray	—	?	Normal	1	2	Dark	?	?	Normal	2	2
8	Gray	?	3	Normal	?	2	Normal	1	?	Normal	2	2
9	Dark	—	—	Normal	—	—	Normal	1	1	Normal	2	2
10	Dark	—	—	Dark	1	?	Brown	1	1	Normal	2	?
11	Dark	—	—	Normal	1	—	Yellow	?	1	Dark	1	2
12	Normal	—	?	Normal	1	?	Normal	1	—	Normal	1	—
13	Normal	1	2	Normal	1	1	Normal	1	—	Normal	2	2
14	Dark	—	?	Normal	?	2	Dark	1	—	Normal	2	2
15	Dark	?	1	Normal	1	1	Normal	?	1	Normal	?	—
16	Normal	?	3	Normal	1	—	Normal	1	—	Normal	2	2
17	Brown	—	—	Normal	—	—	Normal	—	1	Dark	—	—
18	Normal	2	3	Normal	1	4	L. Brown	1	4	Normal	1	1
20	Normal	2	3	Normal	1	2	Normal	1	—	Normal	2	?
21	L Brown	1	2	Normal	1	—	Normal	1	—	Normal	2	2
22	Normal	?	2	Normal	1	1	Normal	1	—	Normal	1	2
23	Brown	—	1	Dark	1	1	Normal	1	—	Dark	—	—
24	Brown	1	1	Dark	1	3	Sl. Dark	1	3	Normal	1	?
25	Normal	1	1	Normal	?	1	Brown	—	—	Normal	1	1
26	Normal	1	1	Normal	1	1	Brown	—	—	Normal	1	1
27	Normal	1	1	Normal	1	1	Brown	—	—	Normal	1	1
28	Dark	—	—	Normal	1	2	Normal	1	—	Normal	1	?
29	Dark	1	1	Normal	1	3	Normal	1	3	Normal	1	?
30	Normal	1	4	Black	1	2	L. Brown	—	—	Normal	—	—
31	Brown	—	2	Normal	1	1	Brown	1	2	Normal	1	—
32	Normal	2	4	Normal	1	3	Normal	1	2	Normal	1	—
33	Dark	—	1	Normal	1	1	Normal	—	—	Normal	1	—
34	L. Brown	?	2	Normal	1	3	Normal	1	1	Normal	1	—
35	L. Brown	?	2	Normal	2	1	Normal	1	—	Normal	—	—
36	Normal	—	—	Normal	1	3	Normal	1	3	Normal	1	—
37	Normal	—	—	Normal	1	2	Normal	—	—	Normal	—	—
38	L. Brown	1	3	Normal	1	3	Normal	1	—	Normal	1	—
39	L. Brown	1	3	Normal	—	3	Normal	—	—	Normal	—	—

Growth on litmus milk showed the production of acid and gas. A few of the cultures reduced the litmus and several caused the formation of a curd.

The softening of vegetables and fruits was studied to some extent. Sweet potatoes failed to show a change as a result of the growth of these organisms. In a few cases gas was produced on apple, but no maceration resulted. The softening of parsnips was less marked than that of potatoes and carrots. The softened condition was the result of a loosening or falling apart of the plant tissue with rarely any destruction of the cell structure. Discoloration of potatoes and carrots was common.

THE CLASSIFICATION OF THE PECTIN-FERMENTING BACTERIA OF THE GENUS *AEROBACTER*

In general, it is probably unwise to use the fermentation of a single sugar or any other single character as a sole basis of specific differentiation. A careful correlation of characters of a dependable nature should be used in place of one or more unrelated differences. The production of indol and acetyl-methyl-carbinol, the liquefaction of gelatin, the use of litmus milk, and the fermentation of many chemically pure sugars have been so standardized as to form reliable bases for the classification of bacteria. The use of pectin as well as other purified natural products should be considered only as an aid to bacterial classification at present. A highly purified pectin may be of value in the future for classifying bacteria when the chemistry and the fermentation of pectin by microorganisms are better understood.

These organisms were found to fall within the genus *Aerobacter* as defined by Weldin (1927). This generic diagnosis is given as follows: "*Motile or non-motile, non-sporeforming rods, fermenting both glucose and lactose with both acid and gas. Produce acetyl-methyl-carbinol (Voges-Proskauer reaction positive); reverse the reaction of* 0.5 *percent glucose-phosphate-peptone solution relatively rapidly; generally able to utilize uric acid as an available source of nitrogen. Pathogenicity usually slight or absent.*"

A study of the members of this genus which were isolated showed them in general to be most closely allied to the three species *Aerobacter cloacae, A. aerogenes,* and *A. oxytocum.* Few only of the forms isolated agree sufficiently with these species to be included with them, the remainder are described as new species. In order to create these new species, other differential characters have been employed in addition to those included in the specific diagnosis by Weldin. It is now necessary to modify the specific diagnoses for *A. cloacae, aerogenes* and *oxyticum* from that given by Weldin in order to differentiate the new from the original species. The modifications suggested are justified by the experimental data at hand.

A careful evaluation and correlation was made of the characters used for classification. It was considered best to establish a new species on not less than two distinct characters. Two cultures differing in one fundamental character or in several minor characters were considered as the same species unless a correlation of characters was not possible. The characters were evaluated on the basis of the purity of the substrate used, the constancy of the results obtained, and on the previous usage of the character in classification.

A key to the organisms has been prepared, using such differential characters as the fermentation of glycerol, dulcitol, aesculin, melezitose, and raffinose and the production of diastase and indol and is outlined below.

Key to the pectin-fermenting bacteria of the genus *Aerobacter*:

a. Acid and gas from glycerol. Non-motile.
  b. Acid and gas from dulcitol.
    c. Acid and gas from melezitose (Nos. 35, 38).....*Aerobacter faeni*
    2c. Neither acid nor gas from melezitose (1, 6, 12, 13, 14, 16, 20, 28, 29, 32, 33, 5, 22, 26, 27, 15, 21, 39)..... *Aerobacter pectinovorum*

  2b. Neither acid nor gas from dulcitol.
    c. Acid and gas from melezitose.
      d. Acid and gas from galactose (7, 9, 10, 11)..... *Aerobacter melezitovorum*

      2d. Neither acid nor gas from galactose (8)..... *Aerobacter diversum*

    2c. Neither acid nor gas from melezitose.
      d. Indol not produced (34).....*Aerobacter aerogenes*

      2d. Indol produced (2, 3, 4, 24, 30, 31) ....*Aerobacter decolorans*

2a. Neither acid nor gas from glycerol. Motile.
  b. Acid and gas from dulcitol.
    c. Indol produced (23) .....*Aerobacter indologenes*

    2c. Indol not produced (25).....*Aerobacter motorium*

  2b. Neither acid nor gas from dulcitol.
    c. Acid and gas from aesculin (18) .....*Aerobacter mitificans*

    2c. Neither acid nor gas from aesculin.
      d. Acid and gas from raffinose (36, 37)..... *Aerobacter salicinovorum*

      2d. Neither acid nor gas from raffinose (17) ..... *Aerobacter pseudoproteus*

The primary groupings of the organisms were made on the basis of glycerol fermentation. This characteristic is correlated with motility and corresponds to the divisions made in previous classifications of the genus *Aerobacter*.

The organisms which were unable to ferment glycerol with the production of acid and gas were in agreement with the diagnosis given by Weldin (1927) for *Aerobacter cloacae*. Weldin's diagnosis is as follows:

"Motile rods, 0.5 to 1.0μ broad by 0.8 to 2.0μ long, conforming to the generic diagnosis. Sucrose is fermented with acid and gas production; glycerol, starch, dulcitol and inositol are rarely attacked and adonitol is not fermented. Gelatin is usually liquefied. Indol is usually produced. Litmus milk is acidified and coagulated. Originally isolated from sewage. Found in the alimentary tract."

In order to subdivide the group, the early descriptions were used. That of Jordan (1890) was brief, but was later confirmed and extended by Castellani and Chalmers (1920). As far as comparison was possible it was found that the later description of *Aerobacter cloacae* differed from the isolated group of non-glycerol fermenters in general by the fermentation of dextrin, the failure to ferment salicin, and by the production of indol. These were the differential characters which justified the formation of a new species for cultures 36 and 37.

Cultures 17 and 18 differed from *Aerobacter cloacae*, in addition to the characters mentioned above for the group, by their failure to liquefy gelatin and to ferment raffinose. Also, culture 17 differed from 18 by the absence of a positive Voges-Proskauer reaction, by the failure to ferment aesculin, and by the ability to macerate vegetables. These organisms have been considered as two new species.

Cultures 23 and 25 also differed from *Aerobacter cloacae* in the same manner as did 36 and 37 and in addition were unable to liquefy gelatin, failed to ferment dextrin, and fermented aesculin and dulcitol. These cultures were differentiated by the production of indol and the fermentation of glycogen, adonitol, and inositol. They were considered as distinct species.

Aerobacter salicinovorum n. sp.

Source: Rotted potato.

Cultures 36 and 37.

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Rods. Arrangement: Single. Limits of size: 0.6μ to 0.8μ by 1.0μ to 2.0μ. Ends: Rounded. Capsules: Absent in twenty-four hours culture of litmus milk.

Endospores: Absent.

Motility: Motile.

Staining reactions: Gram negative. No granular appearance with iodine.

Cultural characteristics:

Colony: Size 2-6 mm. in diameter, round to oval, slightly convex, light grey in color, opaque, no pigment formation on the medium.

Agar streak: Growth abundant, filiform, shiny and butyrous.

Plain broth: Cloudy, ring formation.

Litmus milk: Acid, gas and reduction with curd formation.

Natural media: (36) Softening of carrots. (37) Softening of carrots and parsnips.

Biochemical characters: Indol not formed. Acetyl-methyl-carbinol produced from glucose and sucrose. Nitrates reduced. Uses uric acid as a source of nitrogen. Methyl red reaction is negative. H_2S produced. Gelatin liquefied. (37) Diastase not produced. (36) Diastase produced.

Fermentation reactions Acid and gas from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, rhamnose, trehalose, salicin, xylose, arabinose, mannitol, sorbitol, (36) soluble starch, and pectin (slight by 37). No fermentation from melezitose, amygdalin, aesculin, glycerol, dulcitol, adonitol, inositol, erythritol, dextrin, glycogen, inulin, (37) soluble starch, pentosan, or lignin.

Growth on Endo medium Colony: Medium in size, dark red and of uniform color, slightly convex, nearly flat, and a slightly greenish sheen. (36) Shows no sheen. Medium: Light red—no change.

Growth on eosin methylene blue medium Colony: medium in size, light purple and uniform in color, slightly raised, smooth. Medium: Unchanged.

Diagnosis: *Motile rods, 0.6μ to 0.8μ by 1.0μ to 2.0μ in size, conforming to the generic diagnosis. Acid and gas produced from the hexose sugars, the disaccharides, raffinose, rhamnose, trehalose, salicin, mannitol, sorbitol, and the pentose sugars. Fermentation of pectin variable. No fermentation of melezitose, amygdalin, aseculin, glycerol, dulcitol, adonitol, inositol, erythritol, or of the poly-saccharides. Litmus milk shows acid coagulation and reduction. Indol not formed. Nitrates reduced. Gelatin liquefied. Isolated from rotted potato.*

Aerobacter pseudoproteus n. sp.

Source: Creek water.

Culture 17.

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Short rods. Arrangement: Single and chains. Limits of size: 0.8μ to 2.0μ by 0.6μ to 0.8μ. Ends: Rounded. Capsules: Present in twenty-four hour culture of litmus milk.

Endospores: Absent.

Motility: Motile.

Staining reactions: Gram negative. Granular when stained with iodine.

Cultural characters:

Colony: Size, 2-4 mm. in diameter, round, flat, semi-opaque, amorphous. Medium not browned.

Agar streak: Growth good, filiform, shiny, and butyrous.

Plain broth: Cloudy and pellicle formation.

Litmus milk: Acid and gas.

Natural media: No softening on potato, carrot, parsnip, sweet potato or apple.

Biochemical reactions: Indol not formed. Acetyl-methyl-carbinol produced from salicin. Nitrates reduced. Uses uric acid as a source of nitrogen, sparingly. Methyl red negative. Slight H_2S produced. No liquefaction of gelatin. Diastase not produced.

Fermentation reactions: Acid and gas from glucose, levulose, mannose, galactose, maltose, sucrose, lactose (slight), trehalose, salicin, xylose, arabinose, mannitol, sorbitol, and pectin. No fermentation from raffinose, rhamnose, melezitose, amygdalin, aesculin, glycerol, dulcitol, adonitol, inositol, erythritol, glycogen, dextrin, soluble starch, inulin, pentosan, lignin, or cellulose.

Growth on eosin methylene blue medium Colony: Medium in size, light purple and uniform in color, flat, smooth. Medium: Unchanged.

Growth on Endo medium Colony: Small in size, bronzy to a dark red in color, very flat, showed slight acid production. Medium: Unchanged.

Diagnosis: *Motile rods, 0.8μ to 2.0μ by 0.6μ to 0.8μ in size. Gives a Voges-Proskauer negative reaction, but produces acetyl-methyl-carbinol and thus conforms to the generic diagnosis. Acid and gas produced from mono and di-saccharides, the pentose sugars, trehalose, salicin, mannitol, sorbitol, and pectin. No fermentation from raffinose, rhamnose, melezitose, amygdalin, aesculin, glycerol, dulcitol, adonitol, inositol, erythritol, or the poly-saccharides. Acid and gas from litmus milk. Indol not produced. Nitrates reduced. Gelatin not liquefied. Isolated from creek water.*

Aerobacter mitificans n. sp.

Source: Creek water.

Culture 18.

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Short rods. Arrangement: Single and chains. Limits of size, 1.5μ to 3.5μ by 0.6μ by 0.8μ. Ends: Rounded. Capsules: Present in twenty-four hour culture of litmus milk.

Endospores: Absent.

Motility: Motile.

Staining reactions: Gram negative. Granular with iodine.

Cultural characters: Colony: Size, 2-4 mm. in diameter, round, slightly convex, opaque, shiny amorphous. Medium: Not browned.

Agar streak: Growth abundant, filiform, shiny and butyrous.

Plain broth: Cloudy, with pellicle formation.

Litmus milk: Acid, gas and reduced.

Natural media: Potato, carrot and parsnip softened.

Biochemical reactions: Indol not formed. Acetyl-methyl-carbinol produced from glucose, sucrose, and salicin. Nitrates reduced. Uses uric acid as a source of nitrogen. Methyl red negative. H_2S questionable. No liquefaction of gelatin. Pellicle formed on broth, sediment. Diastase not produced.

Fermentation reactions: Acid and gas from glucose, levulose, mannose, galactose, maltose, sucrose, lactose (slight), trehalose, salicin, aesculin,

xylose, arabinose, mannitol, sorbitol, glycogen and pectin. No fermentation from rhamnose, melezitose, amygdalin, glycerol, dulcitol, adonitol, inositol, erythritol, dextrin, soluble starch, inulin, pentosan, and cellulose.

Growth on Endo medium: Colony: Medium size, red, uniform in color, flat, smooth. Medium: Light pink—no change.

Growth on eosin methelyn blue medium: Colony: Very small, light red, uniform in color, convex, smooth. Medium: No change.

Diagnosis: *Motile rods, 0.6μ to 0.8μ by 1.5μ to 3.5μ in size, conforming to the generic diagnosis. Acid and gas from the mono- and di-saccharides, the pentose sugars, trehalose, salicin, aesculin, mannitol, sorbitol, and pectin. No fermentation from raffinose, rhamnose, melezitose, amygdalin, glycerol, dulcitol, adonitol, inositol, erythritol, or the poly-saccharides. Litmus milk shows a slight reduction with the formation of acid and gas. Gelatin is not liquefied. Indol is not produced. Nitrates are reduced. Shows rapid softening of vegetables. Isolated from creek water.*

Aerobacter indologenes n. sp.

Source: Rotted potato.

Culture 23.

Morphology: Medium: Glucose-phosphate broth. Age: Twenty-four hours. Temperature, 37° C. Form: Short rods. Arrangement: Single and short chains. Limits of size: 0.6μ to 0.8μ by 0.8μ to 2.0μ. Ends: Rounded. Capsules: Present in twenty-four hour cultures of litmus milk.

Endospores: Absent.

Motility: Motile.

Staining reactions: Gram negative. No granular appearance with iodine.

Cultural characters:

Colony: Size 2 mm. in diameter, rounded, raised, translucent, amorphous, and causing the browning of the medium.

Agar streak: Growth good, filiform and butyrous.

Plain broth: Ring, cloudy.

Biochemical reactions: Indol produced. Acetyl-methyl-carbinol produced from glucose and sucrose. Nitrates reduced. Uses urid acid as a source of nitrogen. Methyl red reaction, negative. H_2S produced. Gelatin not liquefied. Skatol produced. Diastase not produced.

Fermentation reactions: Acid and gas produced from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, rhamnose, trehalose, salicin, aseculin, xylose, arabinose, mannitol, dulcitol, sorbitol, adonitol, inositol, glycogen and pectin. No fermentation was produced from melezitose, amygdalin, glycerol, erythritol, dextrin, soluble starch, inulin, pentosan, lignin, and cellulose.

Litmus milk: Acid, gas and reduction with the formation of a curd.

Natural media: Slight softening of potato and carrot.

Growth on Endo medium: Colony: Small in size, dark red and uniform in color, slightly convex but very low, smooth. Medium: Light red.

Growth on eosin methylene blue medium: Colony: Medium in size, light purple and uniform in color, slightly convex.

Diagnosis: *Motile rods, 0.6μ to 0.8μ by 0.8μ to 2.0μ in size, conforming to the generic diagnosis. Acid and gas produced from the common hexose sugars, the di-saccharides, raffinose, rhamnose, trehalose, and the pentose sugars. The alcohols are fermented with the exception of glycerol and erythritol. Pectin is fermented. Acid and gas is produced from many glucosides, but there is no fermentation of the poly-saccharides. Amygdalin is not fermented. Litmus milk is fermented with the production of acid, gas, a coagulation and reduction of the litmus. Indol is produced. Gelatin is not liquefied. Isolated from rotted potato.*

Aerobacter motorium n. sp.

Source: Rotted potato.

Culture 25.

Morphology: Medium: Glucose-phosphate broth. Age: Twenty-four hours. Temperature: 37° C. Forms: Short rods. Arrangement: Single and chains. Limits of size, 0.3μ to 0.5μ by 0.4μ to 1.5μ. Ends: Rounded. Capsules: Present in twenty-four hour culture of litmus milk.

Endospores: Absent.

Motility: Motile.

Staining reactions: Gram negative. Granular appearance with iodine.

Cultural characters:

Colony: Size, 2-4 mm. in diameter, round, raised and slightly convex, semi-opaque, amorphous, with dense center. No pigmentation formed on the medium.

Agar streak: Growth abundant, filiform and shiny and butyrous.

Litmus milk: Acid, gas and reduction. Curd formed.

Natural media: Slight softness on carrot and potato.

Nutrient broth: Cloudy with ring.

Biochemical reactions: Indol not formed. Acetyl-methyl-carbinol produced from glucose, sucrose and mannitol. Nitrates reduced. Uses uric acid as a source of nitrogen. Methyl red negative. H_2S produced. Gelatin slowly liquefied. Skatol not formed. Diastase not produced.

Fermentation reactions: Acid and gas from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, rhamnose, trehalose, salicin, aesculin, xylose, arabinose, mannitol, dulcitol, sorbitol, and pectin. No

fermentation was obtained from melezitose, amygdalin, glycerol, adonitol, inositol, erythritol, glycogen, dextrin, soluble starch, inulin, or pentosan.

Growth on Endo medium: Colony: Small in size, dark red and uniform in color. Slightly convex, smooth and no sheen. Medium: Red.

Growth on eosin methylene blue medium: Small, light purple and uniform in color, slightly convex, smooth. Medium: No change in color.

Diagnosis: *Motile rods, 0.3μ to 0.5μ by 0.4μ to 1.5μ in size, conforming to the generic diagnosis. Acid and gas produced from the common hexose sugars, the di-saccharides, raffinose, rhamnose, trehalose, salicin, aesculin, xylose, and arabinose. The alcohols, mannitol, dulcitol, and sorbitol, are fermented, but glycerol, adonitol, inositol, and erythritol are not fermented. Pectin is fermented. No fermentation occurs from amygdalin or the polysaccharides. Indol is not produced. Gelatin is slowly liquefied. Litmus milk is fermented with the fermentation of acid, gas and a curd with reduction after 3 days. Isolated from rotted potato.*

Aerobacter cloacae

The diagnosis for *Aerobacter cloacae* according to the suggested modification would be as follows: *Motile rods, 0.5μ to 1.0μ broad by 0.8μ to 2.0μ long, conforming to the generic diagnosis. Acid and gas produced from sucrose, maltose, raffinose, galactose, arabinose, and mannitol. No fermentation of glycerol, dulcitol, inositol, adonitol, salicin, and inulin. Gelatin liquefied. Indol is produced. Litmus milk is acidfied and coagulated. Orginally isolated from sewage. Found in the alimentary tract.*

The non-spore forming pectin fermenting bacteria which are able to ferment glycerol may be subdivided on the basis of dulcitol fermentation. Those organisms which do not produce acid and gas from dulcitol conform quite closely to the following diagnosis given by Weldin for *Aerobacter aerogenes. A non-motile rod 0.5 to 0.8μ broad by 1.0 to 2.0μ long, conforming to the generic diagnosis. Acid and gas are formed from sucrose, glycerol, inositol, adonitol, and usually from starch; dulcitol is not attacked. Gelatin is rarely liquefied. Indol is rarely formed. Litmus milk is made acid and coagulated. The organism is found in the alimentary tract of man and animals and widely distributed in nature.*

The study made of these pectin fermenters revealed several distinct groups which gave well correlated characteristics. These groups were distinctly different in several ways, but according to the above diagnosis should be considered as the single species *Aerobacter aerogenes.*

Earlier experimental work on *Aerobacter aerogenes* threw some light upon the question of its classification. One of the characters to be considered with the organisms at hand was that of the production of indol. The work of Castellani and Chalmers (1920) showed the aerogenes organism to be indol negative. However, previous work by MacConkey (1906) gave evidence to show that the organism produced indol. Later extensive studies made of organisms which were roughly considered as aerogenes by Levine and Linton (1924) and by Chen Chong Chen and Rettger (1920) showed that the majority of the organisms isolated from different sources were not able to produce indol. In cases where *aerogenes* was of human origin the percentages of indol positives were much less. The results of MacConkey

might be discounted since earlier tests for indol were apt to give positive rather than negative results due to the nature of the tests employed. It is suggested that *Aerobacter aerogenes* be considered as indol negative. It is further suggested from the results of Levine and Linton (1924) that the failure to ferment melezitose, inulin, and glycogen be considered as a part of the diagnosis for this organism. The suggested diagnosis for *Aerobacter aerogenes* as modified is given as follows:

A non-motile rod, 0.5 to 0.8μ by 1.0 to 2.0μ in size, conforming to the generic diagnosis. Acid and gas are produced from sucrose, maltose, glycerol, inositol, adonitol, mannitol, salicin, and aesculin; dulcitol, inulin, glycogen, and melezitose are not fermented. Gelatin is not liquefied. Indol is not formed. Litmus milk is made acid and coagulated. The organism is found in the alimentary tract of man and animals and widely distributed in nature.

The diagnosis given above is justified on the basis of previous experimental results. This diagnosis permits a better and a more detailed classification for the pectin-fermenting bacteria similar to the *A. aerogenes*. Culture 34 corresponds very closely to the *Aerobacter aerogenes* and it is to be considered thus for the present. Cultures 2, 3, 4, 30, 31, and 24 show a close correlation in characters. These cultures do not correspond to the above diagnosis for the *aerogenes* organism in that they produce indol, do not coagulate litmus milk, and are able to ferment glycogen. They are considered as a distinct species. Cultures 7, 9, 10, 11 differ in a few respects, but they are very closely correlated in the fermentation of melezitose and glycogen, in the formation of indol and in their growth on litmus milk. They are considered as a distinct species. Culture 8 shows several variations from that of the above group of organisms, which makes it a distinct species. This organism is V. P. negative, altho it shows a slight production of acetyl-methyl-carbinol from mannitol. It does not ferment galactose. Melezitose and glycogen are fermented and indol is produced.

Descriptions of these organisms are given as follows, with their assigned name:

Aerobacter decolorans

Culture No. 2, 3, and 24—source, rotted potato; culture No. 4, 30 and 31—Hay infusion.

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Short rods. Arrangement: Single. Limits of size: 0.8μ to 1.2μ by 0.8μ to 5.0μ. Ends: Rounded. Capsules: Abundant in twenty-four hour culture of litmus milk.

Endospores: Absent

Motility: Non-motile.

Staining reactions: Gram negative. Granular with iodine.

Cultural characters:

Colony: Size 2-4 mm. in diameter, round, convex, and arched, opaque, amorphous, not slimy, medium pigmented.

Agar streak: Growth abundant, filiform, shiny, butyrous.

Litmus milk: Acid, or acid and gas.

Plain broth: Cloudy. Ring and sediment.

Natural media: Softening of parsnips, carrots and apple.

Biochemical reactions: Indol produced. Acetyl-methyl-carbinol produced from glucose, sucrose and salicin. Nitrates reduced. Uses uric acid as a source of nitrogen. Methyl red negative, H_2S produced. Gelatin not liquefied. Skatol not produced. Diastase not produced. (24) Produces diastase.

Fermentation reactions: Acid and gas from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, trehalose, salicin, aesculin, xylose, arabinose, glycerol, mannitol, adonitol, inositol, glycogen, soluble starch, and pectin. Acid is formed from rhamnose. Neither acid nor gas produced from melezitose, amygdalin, dulcitol, erythritol, dextrin, inulin, or pentosan.

Growth on Endo medium: Colony: Medium in size, uniformly red in color, slight depression in center, raised, center is darker red and no sheen. Medium: Red. No sheen.

Growth on eosin methylene blue medium: Colony: Large, brownish purple in color, raised to convex, and slimy appearance. Medium: No change.

Diagnosis: *Non-motile rods, 0.6 to 1.0μ broad by 0.8 to 3.0μ long, conforming to the generic diagnosis. Acid and gas from mono- and di-saccharides, the pentose sugars, raffinose, trehalose, salicin, aesculin, many of the common alcohols, glycogen, and pectin. No fermentation from melezitose, amygdalin, dulcitol, erythritol, dextrin, inulin, and pentosan. Acid and gas in litmus milk. Indol is produced. Gelatin is not liquefied. Isolated from rotted potato and hay infusion.*

Aerobacter melezitovorum n. sp.

Source: Special soil.

Cultures 7, 9, 10 and 11.

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Rods with considerable variation in length. Arrangement: Single. Limits of size: 0.8 to 1.2μ broad by 1.0 to 3.0μ long. Ends: Rounded for (7, 9) and truncate for (10, 11). Capsules: Abundant in twenty-four hour culture of litmus milk.

Endospores: Absent.

Motility: Non-motile.

Staining reactions: Gram negative. Slight granulation with iodine.

Cultural characters:

Colony: Size 3-4 mm. in diameter, convex, opaque, radiating strands, (7) tendency to be slimy, amorphous, medium colored, a light or dark brown,

Agar streak: Growth abundant, filiform, shiny and slimy with (7).

Plain broth: Cloudy.

Litmus milk: Acid and gas. No curd.

Natural media: Slight softening of carrots and parsnips.

Biochemical reactions: Indol produced. Acetyl-methyl-carbinol produced from glucose and sucrose. Nitrates reduced. Uses uric acid as source of nitrogen. Methyl red negative. H_2S produced. Gelatin not liquefied. Skatol not produced. Diastase not produced.

Fermentation reactions: Acid and gas from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, rhamnose, trehalose, melezitose, salicin, aesculin, xylose, arabinose, glycerol, mannitol, sorbitol, adonitol, inositol, glycogen, soluble starch, and pectin. Neither acid nor gas from amygdalin, dulcitol, erythritol, dextrin, inulin, and pentosan.

Growth on Endo medium: Colony: Large, uniformly dark red, slightly raised, smooth. Medium: Red. Cultures (7 and 9). Cultures (10 and 11) Colony: Medium to small in size, of uniformly dark red color, slightly defined center, convex and smooth. Usually shows a dark green sheen. Medium: Dark red, showing a green sheen. Very similar in appearance to *Es. coli.*

Growth on eosin methylene blue medium: Colony: Medium in size, purplish to flesh color, slightly raised or convex, smooth, small, dark center with some reverting. Medium showed no change.

Diagnosis: *Non-motile rods, 1.0μ broad and 1.0 to 3.0μ long, conforming to the generic diagnosis. Acid and gas from the mono- and di-saccharides, the pentose sugars, raffinose, rhamnose, trehalose, melezitose, salicin, aesculin, all of the commonly employed alcohols except dulcitol and erythritol, glycogen, soluble starch and pectin. No fermentation from amygdalin, dextrin, inulin and pentosan. Acid and gas in litmus milk. Indol produced. Gelatin not liquefied. Isolated from a special soil mixture.*

Aerobacter diversum n. sp.

Source: Special soil.

Culture 8.

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Short rods. Arrangement: Single or pairs. Limits of size: 1.0μ to 1.2μ by 1.5μ to 3.5μ. Ends: Rounded. Capsules: Largely capsulated in twentq-four hour culture of litmus milk.

Endospores: Absent.

Motility: Non-motile.

Staining reactions: Gram negative. Granular appearance.

Cultural characters:

Colony: Size 5-8 mm. in diameter, round, slightly umbilicate, semi-opaque, finely granular. Medium not discolored.

Agar streak: Growth abundant, filiform, shiny and butyrous.

Plain broth: Cloudy, with slight ring.

Litmus milk: Acid and gas.

Natural media: Softening of potato and carrots.

Biochemical reactions: Indol produced. A slight production of acetyl-methyl-carbinol only from mannitol. Nitrates reduced. Uses uric acid as source of nitrogen. Methyl red negative. H_2S produced. Gelatin not liquefied. Skatol not produced. Diastase produced.

Fermentation reactions: Acid and gas from glucose, levulose, mannose, maltose, sucrose, lactose, raffinose, rhamnose, trehalose, melezitose, salicin, aesculin, xylose, arabinose, glycerol, mannitol, sorbitol, adonitol, inositol, glycogen, soluble starch, and pectin. Acid and gas not produced from galactose, amygdalin, dulcitol, erythritol, dextrin, inulin, and pentosan.

Growth on Endo medium: Colony: Large, dark red in color, dark red center, low, colony but slightly convex, shiny, reddish sheen. Medium: Red.

Growth on eosin methylene blue medium: Colony: Large, pink and uniform in color, slightly figured on surface, raised, and slimy in character. Medium: No change in color.

Diagnosis: *Non-motile rods, 1.0μ broad by 1.0 to 3.0μ long, conforming to the generic diagnosis. Acid and gas produced from the mono- and disaccharides with the exception of galactose. Acid and gas produced from raffinose, rhamnose, trehalose, melezitose, salicin, aesculin, many of the common alcohols, soluble starch, and pectin. No fermentation from amygdalin, dulcitol, erythritol, dextrin, inulin, or pentosan. Acid and gas in litmus milk. Indol produced. Gelatin not liquefied. Isolated from a special mixture of soils.*

The group of non-sporeforming pectin-fermenting bacteria, which ferment glycerol and dulcitol are considered to be related to *Aerobacter oxytocum.* The diagnosis suggested for *Aerobacter oxytocum* by Weldin is given as follows:

"Non-motile rods, conforming to the generic diagnosis; sucrose, dulcitol, glycerol, adonitol, and inositol fermented with the production of acid and gas. Gelatin not liquefied. Indol is usually produced. Litmus milk is acidified and coagulated. Was first isolated from milk. Found in dairy products, soil, and the alimentary tract. Is pathogenic for rabbits on intra-venous injections."

This diagnosis is based on a study of organisms referred to as *oxytocus* by a number of investigators. The primary characteristic used was the fermentation of dulcitol, which was in agreement by all previous investigators. The ability of the organism to produce indol, to liquefy gelatin and to ferment inulin was the source of considerable contradiction by the different investigators. Because of these discrepancies it is probable that the organisms described were different. The organism described by MacConkey (1906), which was obtained from the Kral laboratory at Vienna, may

logically be assumed to be a strain of the original organism isolated by Wyssokowitsch and it agrees with the description given by Flügge (1886) in its failure to liquefy gelatin. By considering the negative liquefaction of gelatin as a character of the original organism, a study of previous investigations show that the failure to produce indol is correlated with this character in most cases. Furthermore, the organism *Bacillus oxytocus perniciosus* (Kral) described by MacConkey does not produce indol. This organism is considered as the type for the species *oxytocum*. The species diagnosis is given as follows:

Non-motile rods, 0.6 to 0.8μ broad by 0.8 to 2.0μ long, conforming to the generic diagnosis. Acid and gas produced from sucrose, raffinose, inulin, salicin, dulcitol, glycerol, adonitol, and inositol. Melezitose and erythritol are not fermented. Gelatin is not liquefied. Indol is not porduced. Litmus milk is acidified and coagulated. Was first isolated from old milk. Found in dairy products, soil, and the alimentary tract. Is pathogenic for rabbits on intravenous injections.

The isolated organisms showed a correlation of indol production with dulcitol fermentation, the failure to produce coagulation on litmus milk, and the fermentation of soluble starch. They do not agree in characters to the diagnosis proposed for *Aerobacter oxytocum*, but do correspond very closely to a number of organisms isolated and studied by MacConkey (1906). These organisms he considered as varieties of the *oxytocus* organism. They are widely distributed in nature, being isolated from milk, soil, decayed vegetables, and possibly from human sources. Levine and Linton (1924) showed results in which reference is made to a group of bacteria designated as group IX, which are probably the same type of organism. These studies indicate the occurrence of a group of organisms which differ from the accepted original *A. oxytocum* organism by the production of indol, the coagulation of litmus milk, and possibly other characters. The isolated forms prove to be capable of fermenting pectin. Cultures 1, 12, 13, 14, 16, 20, 28, 29, 32, 33, and 6, which show a positive diastase reaction, and 5, 15, 22, 26, 21, 27, and 39, which show a negative diastase reaction on starch agar, are considered as organisms in this general group and as a new species. Cultures 35 and 38 are very similar to *Aerobacter melezitovorum* with the exception of the added character—the fermentation of dulcitol—and are considered as a new species.

The descriptions of these organisms with their specific diagnoses are given as follows:

Aerobacter pectinovorum n. sp.

Culture No.	Source
1	Insect
12	Creek water
13	Creek water
14	Creek water
16	Creek water
20	Creek water
28	Creek water
29	Creek water
32	Creek water
33	Creek water
6	Mixed culture
5	Mixed culture
15	Creek water
22	Creek water
26	Mixed culture
27	Mixed culture
21	Creek water
39	Creek water

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Short rods. Arrangement: Single and chains. Limits of size: 0.7 to 1.2μ by 1.0 to 4.0μ. Ends: (13) and (16) truncate others rounded. Capsules: Present in twenty-four hour culture of litmus milk.

Endospores: Absent.

Motility: Non-motile.

Staining reactions: Gram negative. Granules with iodine.

Cultural characters:

Colony: Size 2-3 mm. in diameter, round, opaque to semi-opaque, amorphous. No coloration on medium.

Agar streak: Growth abundant, filiform, shiny and butyrous.

Plain broth: Cloudy with ring.

Litmus milk: Acid and gas. No curd.

Natural media: Softening of potatoes, carrots and parsnips by numbers 15, 21, 32 and 29. Softening of carrots and potatoes by numbers 5, 22, 13, 26, 20, and 27. Softening of carrots by numbers 28, 39, and 14. No softening of vegetables by numbers 6, 12, 33, 16, and 1.

Biochemical reactions: Indol produced. Acetyl-methyl-carbinol from glucose and sucrose. Nitrate reduced. Uses uric acid as source of nitrogen. Methyl red negative. H_2S variable. Gelatin not liquefied. Diastase pro-

duced by numbers 1, 6, 12, 13, 14, 16, 20, 28, 29, 32, and 33. Diastase not produced by numbers 26, 22, 27, 15, 5, 21, and 39.

Fermentation reactions: Acid and gas produced from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, rhamnose, trehalose, salicin, aesculin, xylose, arabinose, glycerol, mannitol, dulcitol, sorbitol, adonitol, inositol, glycogen, dextrin, soluble starch, inulin, and pectin. Neither acid nor gas produced from melezitose, amygdalin, erythritol, and pentosan.

Growth on Endo medium: Colony: Medium, uniformly dark red, raised and convex, slight depression with a center of darker red. Greenish sheen. Medium: Dark red—frequent occurrence of sheen. Often resembling *Es. coli.*

Growth on eosin methylene blue medium: Colony: Medium to large in size, pinkish to purple in color, usually of dark center. Medium: Unchanged. Often resembling *Aerobacter aerogenes.*

Diagnosis: *Non-motile rods, 0.8μ broad and 1.0 to 3.0μ long, conforming to the generic diagnosis. Acid and gas from the mono- and di-saccharides, pentose sugars, raffinose, rhamnose, trehalose, salicin; aesculin, glycerol, dulcitol, and other alcohols, but not erythritol, glycogen, most poly-saccharides, and pectin. No fermentation from melezitose, amygdalin, or pentosan. Acid and gas produced in litmus milk. Indol is produced. Gelatin is not liquefied. Isolated from creek water.*

Aerobacter faeni n. sp.

Cultures No.	Source
35	Hay infusion
38	Hay infusion

Morphology: Medium: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Short rods. Arrangement: Single and chains. Limits of size 0.8 to 1.5μ by 1.0 to 3.0μ. Ends: Rounded. Capsules: Present in twenty-four hour culture of litmus milk.

Endospores: Absent.

Motility: Non-motile.

Staining reactions: Gram negative. Granular with iodine.

Cultural characters:

Colony: Size 2-4 mm. in diameter, round, convex, opaque, shiny, amorphous. No coloration of medium.

Agar streak: Growth abundant, filiform, shiny and butyrous.

Plain broth: Cloudy.

Litmus milk: Acid and gas.

Natural media: Softening of potatoes and slight softening of carrots and parsnips.

Biochemical reactions: Indol produced. Acetyl-methyl-carbinol produced from glucose and sucrose. Nitrates reduced. Uses uric acid as a source of nitrogen. Methyl red negative. H_2S produced. Gelatin not liquefied. Skatol not produced. Diastase not produced.

Fermentation reactions: Acid and gas produced from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, rhamnose, trehalose, salicin, aesculin, xylose, arabinose, glycerol, mannitol, dulcitol, sorbitol, adonitol, inositol, glycogen, soluble starch melezitose, and pectin. Acid and gas not produced from inulin, dextrin, amygdalin, erythritol, or pentosan.

Growth on Endo medium: Colony: Small to medium in size, uniformly dark red in color, nearly flat, slightly depressed center, and bluish sheen. Medium: Dark red.

Growth on eosin methylene blue medium: Colony: Small. Light purple, raised or convex. Medium: Color unchanged.

Diagnosis: *Non-motile rods, 1.0μ broad and 1.0 to 3.0μ long, conforming to the generic diagnosis. Acid and gas produced from the mono- and disaccharides, including melezitose, from pentose sugars, raffinose, rhamnose, trehalose, salicin, aesculin, all the alcohols except erythritol, from glycogen, soluble starch, and pectin. No fermentation from amygdalin, inulin, or the pentosans. Acid and gas in litmus milk. Indol produced. Gelatin not liquefied. Isolated from hay infusion.*

STUDIES ON THE PECTIN FERMENTING BACTERIA BELONGING TO THE GENUS *BACILLUS*

This group of organisms was studied by the same procedure employed for those of the genus *Aerobacter*.

The results of the detailed study of these organisms are included in Table II.

TABLE II. Cultural Reaction of Pectin-Fermenting Bacteria of the Genus Bacillus.

Culture	Shape	Size in μ		Arrangement	Gram reaction	Granules		Capsules
No.		Wide	Long			Iodine	M. B.	
40	L. rod	0.3-0.5	2.0-3.5	S. Chain	—	—	—	—
41	L. rod	0.4-0.5	2.0-3.5	S. Chain	—	—	—	—
*								
172	L. rod	0.5-0.6	1.5-3.0	S. Chain	—	+	—	
173	L. rod	0.6-0.7	3.0-4.0	S. Chain	—	+	—	

*Culture numbers 172 and 173 are strains of *Bacillus aceto-ethylicum*.

L. rod = Long rod
+ = Positive reaction
— = Negative reaction
+r = Acidity followed by reduction
? = Questionable
A = Acid
G = Gas
Gl = Glucose
Su = Sucrose
M = Mannitol
Sa = Salicin
1, 2, 3, 4 = Proportional activity
N = Normal
S = Softened
S. Chain = Single and chains

TABLE II—(Continued)

Culture	Spores	Motility	Indol	Acetyl-Methyl-Carbinol				Nitrate reductions	Uric Acid	M. R. reaction
No				Gl	Su	M	Sa			
40	+	+	—	—	—	—	—	+	+	—
41	+	+	—	—	—	—	—	+	1	+
*										
172	+	+	—	—	—	—	—	+	1	+
173	+	+	—	—	—	—	—	+	1	+

*Culture numbers 172 and 173 are strains of *Bacillus Aceto-ethylicum*.

TABLE II—(Continued)

Culture	H_2S	Proteolysis	Skatol	Source	Broth	Litmus Milk				Diastase
No						A	G	R	C	
40	—	+	—	Cornstalks	?	+	+	+	—	+
41	—	+	—	Cornstalks	?	+	+	+	—	+
*										
172	—	—	—	Stock	Cloudy	+	+	+	—	+
173	—	—	—	Stock	Cloudy	+	+	+	—	+

*Cultures Number 172 and 173 are strains of *Bacillus aceto-ethylicum.*

TABLE II—(Continued)

Culture	Glucose		Levulose		Mannose		Galactose		Maltose		Sucrose		Lactose	
No.	A	G	A	G	A	G	A	G	A	G	A	G	A	G
40	+r	+	+r	+	+r	+	+r	+	+	+	+r	+	+r	+
41	+r	+	+r	+	+r	+	+r	+	+	+	+r	+	+r	+
*														
172	+	+	+r	+	+r	+	+r	+	+	+	+	+	+r	+
173	+	+	+r	+	+r	+	+r	+	+	+	+	+	+r	+

*Cultures Number 172 and 173 are strains of *Bacillus aceto-ethylicum.*

TABLE II—(Continued)

Culture	Raffinose		Rhamnose		Trehalose		Melezitose		Salicin		Amygdalin		Aesculin		Xylose	
No.	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
40	+r	+	+r	+	+	+	+	+	+	+	+	—	+	+	+	+
41	+r	+	+r	+	+	+	+r	+	+	+	+	—	+	+	+r	+
*																
172	+r	+	+r	+	+	+	+r	+	+	+			+r	+	+r	+
173	+r	+	+r	+	+	+	+r	+	+	+			+r	+	+r	+

*Cultures Number 172 and 173 are strains of *Bacillus aceto-ethylicum.*

TABLE II—(Continued)

Culture	Arabinose		Glycerol		Mannitol		Dulcitol		Sorbitol		Adonitol		Inositol		Erythritol	
No.	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
40	+r	+	+r	+	+r	+	—	—	+r	+	—	—	—	—	+	+
41	+r	+	+r	+	+r	+	—	—	+r	+	—	—	—	—	+	?
*																
172	+	+	+	+	+	+	?	—	+	+	—	—	—	—	+	—
173	+r	+	+r	+	+	+	?	—	+r	+	—	—	—	—	+	?

*Cultures Number 172 and 173 are strains of *Bacillus aceto-ethylicum.*

Table II—(Continued)

Culture	Glycogen		Dextrin		Sol. Starch		Inulin		Pectin		Pentosan		Lignin		Cellulose	
No.	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
40	+	+	+r	+	+	+	+r	+	+	+	+r	+	—	—	—	—
41	+	+	+r	+	+r	+	+r	+	+	+	+r	+	—	—	—	—
*																
172	+	+	+r	+	+	+	?	+	+	+	+r	+				
173	+	+	+r	+	+	+	?	+	+r	+	+r	+				

*Cultures Number 172 and 173 are strains of *Bacillus acetoethylicum.*

Table II—(Continued)

Culture	Potato			Carrot			Parsnip			Apple		
No.	Color	G	S	Color	G	S	Color	G	S	Color	G	S
40	Normal	1	3	Normal	2	4	Normal	—	—	Normal	3	4
41	Normal	1	3	Normal	2	4	Normal	—	—	Normal	2	4
172	Normal	1	3				Normal	—	—			
173	Normal	1	3				Normal	1	2			

*Cultures Number 172 and 173 are strains of *Bacillus acetoethylicum.*

The pectin-fermenting bacteria of the spore-forming, aerobic type which were isolated and studied are very similar to the organism *Bacillus aceto-ethylicum*. The only significant difference observed was the ability to liquefy gelatin.

Detailed study of these organisms revealed their inability to produce indol or acetyl-methyl-carbinol. They are further differentiated from the aerobic, non-sporeforming pectin fermenters by their ability to ferment pentosan. This latter characteristic may explain the ability of these organisms to ret cornstalks. The retting proceeds very slowly, starting with the certical layer of the slices of cornstalks. However, lignin and cellulose are not fermented.

A consideration of these organisms from the standpoint of their classification was first undertaken by the detailed study of strains 172 and 173 of *Bacillus aceto-ethylicum*. The results of this study show a very close agreement to the original *Bacillus aceto-ethylicum* described by Northrop (1919). The isolated cultures, with the exception of the ability to liquefy gelatin, are nearly identical with the former. Further differences were observed in the colony growth and in the type of maceration produced. The isolated forms completely destroyed all cellular tissue with the exception of the tracheal tubes. However, the effect of *Bacillus aceto-ethylicum* on potato is a softening and not so much a complete destruction.

These two related organisms seem to be very similar to *Bacillus macerans* as described by Schardinger (1897) and to *Bacillus asterosperous* described by Northrop, Ashe and Senior (1919). Beijerinck and den Dooren de Jong (1923) considered *Bacillus asterosporus* and *Bacillus comesii* and other similar retting and pectin fermenting organisms to be grouped under the name *Bacillus polymyxa*.

DESCRIPTION OF THE PECTIN FERMENTING BACTERIA BELONGING TO THE GENUS *BACILLUS*

The two cultures isolated differ only in their ability to grow on solid media. The description applies to both cultures.

Bacillus aceto-ethylicum

Source: Cornstalk material.

No. 40 and 41.

Morphology: Glucose-phosphate-peptone broth. Age: Twenty-four hours. Temperature: 37° C. Form: Long, slim rods. Arrangement: Single or in short chains. Limits of size: 2.0μ to 3.5μ by 0.3μ to 0.5μ. Capsules: Not present in twenty-four hour cultures of litmus milk.

Endospores: Present-terminal.

Motility: Motile.

Staining reactions: Gram negative. No granules appeared with iodine.

Cultural characters:

Colony: Small, with a tendency toward spreading, grumose, edge-lobate, cochleate form, flat and clear.

Agar streak: Scant growth, beaded with lobated border.

Plain broth: Scant turbidity, no pellicle or ring.

Litmus milk: Acid and excessive gas production.

Natural media: Complete destruction of potato, carrot, parsnip, and apple tissue.

Biochemical reactions: Indol not produced. Acetyl-methyl-carbinol not produced. Nitrate reduction. Methyl red: Indefinite. H_2S not produced. Gelatin liquefied. Diastase produced. Uses uric acid as a source of nitrogen, sparingly.

Fermentation reactions: Acid and gas produced from glucose, levulose, mannose, galactose, maltose, sucrose, lactose, raffinose, rhamnose, melezitose, salicin, aesculin, xylose, arabinose, glycerol, mannitol, sorbitol, glycogen, dextrin, soluble starch, inulin, pectin, and pentosan. Slight fermentation was recorded from amygdalin and erythritol. Acid and gas not produced from dulcitol, adonitol, inositol, lignin, and cellulose.

Growth on Endo and E. M. B. media: Colonies were very small, convex and indicated very little change in the medium.

Remarks: Until further need for the separation of these organisms is evident, it is thought best to consider them as strains of *Bacillus acetoethylicum*.

SUMMARY AND CONCLUSIONS

The studies represented by this investigation have led to the differentiation of new species of bacteria which are capable of fermenting pectin. The organisms which were isolated and found to be pectin fermenting bacteria fall in three genera; Aerobacter, Bacillus, and Clostridium. These pectin fermenters are widely distributed in nature and are commonly associated with the decay of plant tissue.

The purified pectin employed, when used in a medium, has proven satisfactory for the isolation and identification of pectin-fermenting bacteria. It is suggested that further study of the fermentation of this purified pectin may throw some light on the chemistry of pectin as well as on the systematic relationships of the organisms.

The pectin-fermenting bacteria, as a group, are characterized by active starch fermentation, the presence of granules when stained with iodine, the ability to ferment a large number of sugars, alcohols, glucosides, and polysaccharides, and the ability to disintegrate plant tissue.

BIBLIOGRAPHY

ALLEN, PAUL W.
1918. A simple method for the classification of bacteria as to diastase production. Jour. Bact., 3:15-17.

AHMANN, C. F. AND HOOKER, H. D.
1925. The estimation of pectin and a study of the constitution of pectin. Research Bulletin 77, Missouri Ag. Exp. Sta.

BEHRENS, J.
1902. Untersuchungen über die Gewinnung der Hanffaser durch natürliche Röstmethoden. Centralbl. f. Bakt. Abt. II, 8:117-120, 131-137, 161-166,202-210, 231-236.

BEIJERINCK, M. W. AND VAN DELDEN, A.
1903. Over de bacterien, wilke bij het roten van vlas werdsaam zijn. Kon. Akad. Wetensch. te Amsterdam, 19:673.
1904. Sur les bactéries active dans le rouissage du lin. Arch. Sci. Ex. Nat. Neerl. (II), 9:418-441.

*BEIJERINCK, M. W. AND L. E. DEN DOOREN DE JONG.
1923 K. Akad. Wetensch., Amsterdam, Proc. Sect. Sci., 25:279.

BERGEY, DAVID H.
1925. Bergey's manual of determinative bacteriology. The Williams and
COMMITTEE ON NOMENCLATURE OF PECTIN.

BERTRAND, G. AND MALLÈVRE, A.
1894. Sur la pectase et sur la fermentation pectique. Compt. Rend. Acad. Sci. Par., 119:1012-1014.

BOURQUELOT, E. AND HERISSEY, H.
1898. Sur l'existence, dans l'orge germée, d'un ferment soluble agissant sur la pectine. Compt. Rend. Acad. Sci. Par., 127:191-194.

BRACONNOT, M. HENRI.
1825. Recherches sur un nouvel acide universellement répandu dans tous les végétaux. Ann. Chim. et Phys., 28:173-178.

CARBONE, D. AND TOBLER, F.
1922. Die Röste mit Bacillus felsineus. Faserforschung, 2:163-183.

CARRÉ, M. H. AND HAYNES, D.
1922. XII. The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. Biochem. Jour., 16:60-69.

CASTELLANI, ALDO AND CHALMERS, ALBERT J.
1920. Sur la classification de certains groupes de bacilles aérobes de l'intestin humain. Ann. Inst. Pasteur. 34:600-622.

CHEN CHONG CHEN AND RETTGER, LEO F.
1920. A correlation study of the colon-aerogenes group of bacteria, with special reference to the organisms occurring in the soil. Jour. Bact. 5:253-299.

COLES, HAROLD W.
1926. The digestion of pectin and methylated glucoses by various organisms. Plant Physiology, 1:379-386.

COMMITTEE ON NOMENCLATURE OF PECTIN.
1927. Definitions written by the Committee on Nomenclature of pectin of the Agriculture-Food Division. Am. Chem. Soc. Jour., 49:(Pro.) 37-39.

*CORRENS, ERICH.
1921. (The knowledge of pectin substances of flax.) Faserforschung, 1:229-240.

DONKER, H. J. L.
1926. Thesis. Bijdrage tot de kennis der boteruur-butytalcoholen acetongistingen. W. D. Meinema, Delft.

DORE, W. H.
1926. The pectic substances. Jour. Chem. Ed., 3:505.

*DUGGELI, M.
1921. Die Bakterien des Waldobens. Schweiz. Ztschr. f. Forstwesen 1923; Forchungen suf dem Gebiete der Bodenbakteriologie. Landw. Vorträge. Verlag Ruber, Frausenfeld. 1921. H. 3.

EHRLICH, FELIX.
1917. Die Pektinstoffe, ihre Konstitution und Bedeutung. Chem. Ztg., 41: 197-200.
1927. Neue Untersuchungen über Pektinstoffe. Ztschr. Agnew. Chem., 40:305-313.

VON FELLENBERG, TH.
1918. Uber die Konstitution der Pektinkörper. Biochem. Zeit., 85:118.

FLUGGE, C.
1886. Die Mikroorganismen F. C. W. Vogel, Leipzig. p. 268.

FRAZIER, WILLIAM C.
1926. A method for the detection of changes in gelatin due to bacteria. Jour. Inf. Dis., 39:302.

FREMY, M. E.
1848. Memoire sur la maturation des fruits. Ann. de Chim. et Phys. (3) 24:5-58.

HARDING, H. A. AND STEWART, F. C.
1902. A bacterial soft rot of certain cruciferous plants and Amorphophallus simlinense. Science, 16:314.

HARTER, L. L. AND WEIMER, J. L.
1921. A comparison of the pectinose produced by different species of Rhizopus. Jour. Ag. Research, 22:371-377.

JONES, L. R.
1901. Concerning Bacillus carotovorus and softening of plant tissue. Centralbl. f. Bakt. Abt. II, 7:12,61.
1905. The cytolytic enzyme produced by Bacillus carotovorus and certain other soft rot bacteria. Centralbl. f. Bakt. II, Abt., 14:910.

JORDAN, EDWIN O.
1890. A report of certain species of bacteria observed in sewage. Report on Water Supply and Sewage, Experimental Investigations by the State Board of Health of Massachusetts. Part II, p. 821.

KOLB, M. J.
1868. Recherches sur le blanchiment des tissus. Compt. Rend. Acad. Sci. Par., 66:1024.

KRUSE, W.
1910. Allgemeine Mikrobiologie. F. C. W. Vogel, Leipzig, p. 226.

LEVINE, MAX AND LINTON, CLAIR S.
1924. Differentiation of human and soil strains of the aerogenes section of the colon group. Am. Jour. of Public Health. 14:95-100.

MACCONKEY, ALFRED.
1906. A contribution to the bacteriology of milk. Jour. Hyg., 6:385-407.

MAKRINOW, I. A.
1915. Sur un nouveau microorganisme provoquant la fermentation de l'amidon et des matiéres pectiques. Arch. Sci. Biol., 18:305.

MANGIN, L.
1889. Sur la présence des composés pectiques dans les végétaux. Compt. Rend. Acad. Sci. Par., 109:579-582.
1891. Etude, historique et critique sur la présence des composés pectiques dans les tissus des végétaux. Jour. De. Botan., 5:400-413.
1893. Sur l'emploie du rouge de ruthénium en anatomie végétale. Compt. Rend. Acad. Sci. Par., 116:653-656.

MEYER, ARTHUR.
1897. Studien uber die Morphologie and Entwickelungsgeschichte der Bacterien, ausgeführt an Astasia asterospora A. M. und Bacillus tumescens Zopf. Flora, 84:14.

NANJI, D. R., PATON, F. J. AND LING, A. R.
1925. Decabonization of polysaccharide acids: Its application to the establishment of the constitution of pectins and to their determination. Jour. Soc. Chem. Ind., 44:253T.

NORRIS, FREDERICK W. AND SCHRYVER, SAMUEL B.
1925. The Pectic Substances of Plants. Part III. The nature of pectinogen and its relation to pectic acid. Biochem. Jour., 19:676-693.

NORTHROP, JOHN H., LAUREN H. ASHE AND JAMES K. SENIOR.
1919. Biochemistry of bacillus acetoethylicum with reference to the formation of acetone. Jour. Biol. Chem. 39:1-2.

ORLA-JENSEN, S.
1919. Aerobes and facultative anaerobes. Centralbl. f. Bakt., II Abt., 22:305.

PAYEN, M.
1856. Note sur la racine charnue du cerfeuil bulbeaux Compt. Rend. Acad. Sci., Par., 43:769-775.

RITTER, GEORGE J.
1925. Distribution of lignin in wood. Ind. and Eng. Chem. 17:1194-1196.

*RITTER, GEORGE J. AND FLECK, L. C.
1923. A paper read at Washington meeting of Amer. Chem. Soc., April, 1924. Unpublished.

*ROSSI, G.
1916. Bull. Agric. Intell., 7:1067.

SCHARDINGER, FRANZ.
1897. Bacillus macerans, ein Aceton Bildender Rotte-bacillus. Centralbl. f. Bakt. Abt. II. 14:772-782.

STÖRMER, K.
1904. Uber die Wasseroste des Flachses. Centralbl. f. Bakt. II Abt., 13:35-45.

SUCHARIPA, R.
1924. Protopectin and some other constituents of lemon peel. Jour. Am. Chem. Soc., 46:145.

TOLLENS, B.
1895. Ueber die Constitution der Pectinstoffe. Liebig's Annal., 226:292-295.

*TSCHIRCH, A.
1908. Ueber die Pektinmetamorphose, Inauguraldissertation. Bern.
1910. Handbuch der Pharmakognosie. C. H. Tauchnitz, Leipzig (1910-14). pp. 399-400.

VAN TIEGHEM, M.
1877. Sur le Bacillus amylobacter et son role dans la putréfaction des tissus végétaux. Bull. de Soc. Botan. de France, 24:128-135.

VAN WISSELINGH, C.
1898. Mikrochemische untersuchungen uber die zellwande der fungi. Jahrb. wiss. Botan., 31:618-667.

WAKSMAN, SELMAN A.
1927. Principles of soil microbiology. The Williams and Wilkins Co., Baltimore, p. 38.

WELDIN, JOHN C.
1927. The colon-typhoid group of bacteria and related forms. Relationships and classifications. Iowa State College Journal of Science, 1:121-197.

WEYER, ELLIOTT R. AND RETTGER, LEO F.
1927. A comparative study of six different strains of the organisms commonly concerned in large-scale production of butyl-alcohol and acetone by the biological process. Jour. Bact. 14:399-424.

WINOGRADSKY, S. AND FRIBES, V.
1895. Sur le rouissage du lin et son agent microbien. Compt. Rend. Acad. Sci. Par., 121:242.

*Original not seen.
*Original not seen.
*Original not seen.

SUCTORIA OF THE LARGE INTESTINE OF THE HORSE: *ALLANTOSOMA INTESTINALIS* GASSOVSKY, *A. DICORNIGER* SP. NOV., AND *A. BREVI-CORNIGER* SP. NOV.

TA-SHIH HSIUNG

From the Zoological Laboratory, Iowa State College.

Accepted for publication Oct. 1, 1928.

In the course of a microscopic investigation of the protozoa which inhabit the large intestine of the horse at least three distinct species of Suctoria were observed. Up to the present time the writer has examined fifteen horses which were sacrificed for the Operative Surgery and the Post-mortem Technique classes of the Division of Veterinary Medicine of Iowa State College. Fresh feces of several other horses were also examined.

In the material taken from the colon of horse No. 6, the writer noted a suctorian (Fig. 1) which agrees with the general description of *Allantosoma intestinalis* Gassovsky, except for some minor differences. On two other occasions this suctorian was found in the fresh feces of horses. Gassovsky states that *A. intestinalis* bears one to seven suckers on each end of the body, but in the material from none of these three horses has the writer found even one specimen bearing only one sucker on each end of the body. So far as the writer can find, the number of suckers on either end of *A. intestinalis* varies from three to seven. The measurements as given by Gassovsky for *A. intestinalis* are 16-65μ x 5-27μ. While the length of the body of *A. intestinalis*, measured by the writer, varies from 33-60μ, with a mean length of 47.7μ, the width of the body varies from 18-37μ, with a mean width of 26μ. The diameter of the macronucleus is about 10μ. Some of them were attached to the bodies of parasitic ciliates *Cycloposthium bipalmatum* and *Blepharocorys curvigula*.

In the material taken from the colon of horse No. 12, the writer noted a second species of Suctoria. This new suctorian (fig. 2) differs from *A. intestinalis* mainly by its smaller macronucleus as well as by its smaller body size and its consistency in bearing only one tentacle on each end of the body. It should be noted here that not a single specimen of *A. intestinalis* type was present in the material in which this new suctorian was found. The tentacles of this new suctorian, which are bowed toward each other on the ends of the body, suggest in appearance a pair of horns. Therefore the name *Allantosoma dicorniger* is suggested for this new species of Suctoria. The body is more or less sausage-shaped, bearing one incurved tentacle on each end. The outline of the distal end of the tentacle somewhat resembles that of a boot. The surface which lies between these two tentacles is nearly flat, while the remaining surface is convex. The cytoplasm, as in *A. intestinalis*, is filled with numerous refractile granules. A more or less spherical macronucleus with a mean diameter of 6μ is located near the center of the body. A small spherical micronucleus lies by the side of the macronucleus. A single contractile valuole can usually be seen near the

macronucleus. The length of the body of this new suctorian varies from 20-33μ, with a mean length of 27μ; the width of the body varies from 10-20μ, with a mean width of 16.4μ. The ratio between the length and the width of the body ranges from 1.2-1. to 2.5-1, with a mean ratio of 1.6-1. It has been found only in the colon. Every individual observed was unattached to any other organism.

In the material taken from the caecum of horse No. 15, the writer noted still a third species. At the first glance this suctorian resembles *A. dicorniger* in general form, but upon closer and more critical examination there are to be found several important features which separate this suctorian from *A. dicorniger*. Not a single specimen which could be identified as either *A. intestinalis* or *A. dicorniger* was present together with this species. Because of its short tentacles, the name *Allantosoma brevi-corniger* is suggested for this new species. The body of this new suctorian (fig. 3) resembles that of an elongated *A. dicorniger*. It also bears one slightly incurved tentacle on each end of the body. The tentacles are shorter and more slender than those of *A. dicorniger*. The sucker, which is at the distal end of the tentacle, does not form a boot-shaped expansion as does that of *A. dicorniger*. Another difference is that the cytoplasm of this species is nearly homogeneous, while that of *A. dicorniger* and *A. intestinalis* contains numerous refractile granules. An oval macronucleus, with a mean length of 6.8μ and a mean width of 4.4μ, is usually located in the center of the body. A small spherical micronucleus lies by the side of the macronucleus. A single contractile vacuole is usually located near the macronucleus. The length of the body of this new suctorian ranges from 23-36μ, with a mean length of 29.6μ; the width of the body ranges from 7-11μ, with a mean width of 8.6μ. The ratio between the length and the width of the body ranges from 2.4-1 to 4.1-1, with a mean ratio of 3.4-1 as compared to 1.6-1 in *A. dicorniger*. It is often found attached to the body of the ciliate *Paraisotricha colpoidea* by one tentacle. It has been found only in the caecum so far.

REFERENCE CITED

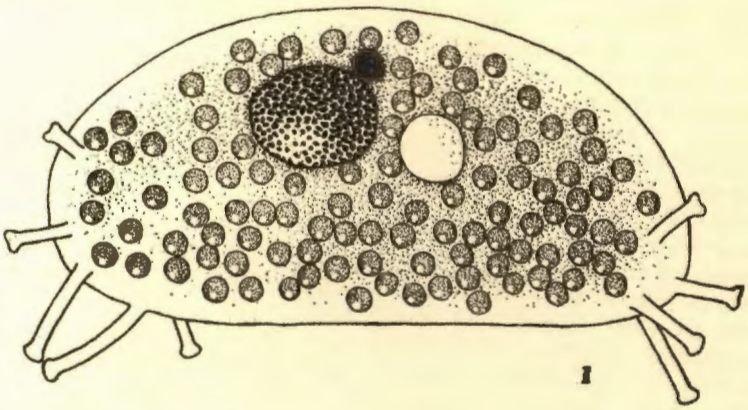
GASSOVSKY, G.
1919. Notes et Communications. On the microfauna of the intestine of the horse. Travaux de la Soc. des Naturalistes de Pétrograd (1918). 49:20-37. English Translation, 65-69.

EXPLANATION OF PLATE

Fig. 1. *Allantosoma intestinalis* Gassovsky. Magnification X 1707.

Fig. 2. *Allantosoma discorniger* sp. nov. Magnification X 1707.

Fig. 3. *Allantosoma brevi-corniger* sp. nov. Magnification X 1707.



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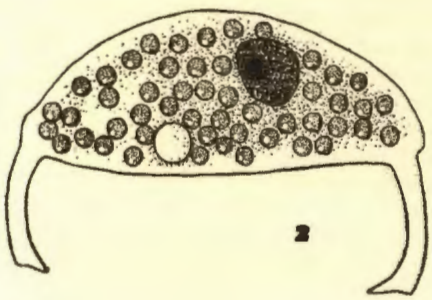

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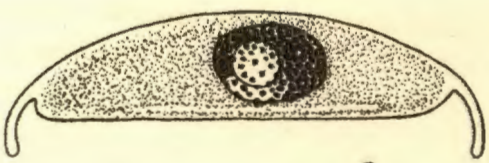

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